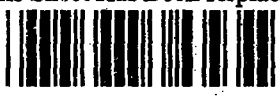
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T CELL ANTIGEN RECEPTOR V REGION PROTEINS AND METHODS OF PREPARATION THEREOF

INTRODUCTION

The present invention is generally in the fields of immunology and medicine and relates to full length recombinant T cell receptor (TCR) V region proteins, expression vectors for producing commercially useful amounts of recombinant T cell antigen receptor V region proteins, and methods of using full length recombinant T cell receptor V region proteins to diagnose and treat diseases or disorders of the immune system.

BACKGROUND OF THE INVENTION

1. THE T CELL ANTIGEN RECEPTOR

The immune response to foreign antigens is divided into humoral immunity, mediated by B cells, and cellular immunity, mediated by T cells. The receptor molecules used by B cells to recognize antigen are immunoglobulins which recognize conformational epitopes on globular protein. The antigen receptors on T cells are related to immunoglobulins, but bind processed antigen molecules in association with self major histocompatibility complex (MHC) molecules (Dembic et al., 1986, *Immunol. Today*, 7:308).

TCR genes, like immunoglobulin genes, consist of coding regions (or gene segments) which rearrange during T cell ontogeny (Chien et al., 1984, *Nature*, 312:31-35; Hedrick et al., 1984, *Nature*, 308:149-153; Yanagi et al., 1984, *Nature*, 308:145-149). The human T cell receptor ß chain locus has been extensively studied since the cloning of the first cDNA encoding the ß-chain (Yanagi, Y., et al. 1984, *Nature*, 308:145-149; Hedrick et al., 1184, *Nature*, 308:149-152; Siu et al., 1984, *Cell*, 37:393-401). This locus is a gene complex comprising regions encoding known regions or domains of the β chain: the variable (V), diversity (D), and joining (J) domains. These coding regions participate in somatic cell rearrangement with a region encoding the constant (C) domain (Chien et al., 1984, *Nature*, 309:322-326). *In situ* studies identified the location of the TCRß locus on chromosome 7 at 7q35 (Isobe et al., 1985, *Science*, 228:580). By current estimates, this complex spans more than 600 kb and contains 70 to 80 variable region segments (Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Tillinghast et al., 1986, *Science*, 233:879-883; Kimura et al., 1987, *Eur. J. Immunol.*, 17:375-383; Lai et al., 1988, *Nature*, 331:543-546). These V region gene segments are adjacent to two tandemly organized regions, each of which includes a D and a C gene segment followed by a cluster of six or seven J region gene segments (Tunnacliffe et al., 1985, *Nucleic Acids Res.*, 13:6651-6661); Toyonaga et al., 1985, *Proc. Natl. Acad. Sci. USA*, 82:8624-8628). Following successful DNA rearrangement of the V, D, and J coding regions (gene segments), the translated ß chain polypeptide pairs with a TCR α chain and can be expressed on the surface of the T cell (reviewed by Kronenberg et al., 1986, *Ann. Rev. Immunol.* 4:529-591)

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The TCR α and δ locus are next to one another on human chromosome 14. TCR δ coding regions are located entirely within the α gene locus (Satyanarayana et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:8166-8170; Chien et al., 1987, *Nature*, 330:722-727; Elliott et al., 1988, *Nature*, 331:627-631). It is estimated that there are a minimum of 45-50 Vα coding regions (Becker et al., 1985, *Nature*, 317:430-434), whereas there are only approximately 10 Vδ coding regions (Chien et al., 1987, *supra*). In peripheral blood, two Vδ coding regions appear to be predominantly expressed, namely, Vδ 1 and Vδ 2.

The γ TCR gene was identified, first in mice (Saito et al., 1984, *Nature*, 309:757-762; Kranz et al., 1985, *Nature*, 313:762-755; Hayday et al., 1985, *Cell*, 40:259-269) and then in humans (Lefranc et al., 1985, *Nature*, 316:464-466; Murre et al., 1985, *Nature*, 316:549-552). The human γ TCR locus appears to consist of between five and ten V, five J and two C coding regions (Dialynas et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:2619).

TCR diversity, and thereby T cell specificity, is derived from several sources (Barth et al., 1985, *Nature*, 316:517-523; Fink et al., 1986, *Nature*, 321:219-225): the multiplicity of germline gene segments (Chien et al., 1984, *Nature*, 309:322-326; Malissen et al., 1984, *Cell*, 37:1101-1110; Gascoigne et al., 1984, *Nature*, 310:387-391; Kavaler et al., 1984, *Nature*, 310:421-423; Siu et al., 1984, *Nature*, 311:344-349; Patten et al., 1984, *Nature*, 312:40-46); combinatorial diversity through the assembly of different V, D, and J coding regions (Siu et al., 1984, *Cell*, 37:393-401; Goverman et al., 1985, *Cell*, 40:859-867); and junctional flexibility, N-region diversity and the use of either multiple D regions or any of the three translational reading frames for Dß segments. TCR diversity does not appear to arise from the somatic hypermutation mechanism observed for immunoglobulins (Barth et al., *supra*). As a result of these mechanisms, TCRs are generated which differ in their amino-terminal, or N-terminal, domains (i.e., are constructed from combinations of V, D, and J coding regions) but are similar elsewhere, including their carboxyl-terminal, or C-terminal domains (which constitute constant regions of TCRs). Accordingly, an extremely large repertoire of TCR molecules is generated.

Study of the structure and diversity of the human TCR ß-chain variable coding regions has led to the grouping of these coding regions into distinct V region subfamilies (Tillinghast et al., 1986, *Science*, 233:879-883; Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Borst et al., 1987, *J. Immunol.*, 139:1952-1959). Each V region subfamily can be characterized by a consensus sequence and contains similar coding regions exhibiting greater than about 75% similarity in the DNA sequence. For instance, sixteen mouse Vß sequences have been categorized into fourteen different subfamilies by a proposed arbitrary, but simple, numerical nomenclature for the Vβ gene segments (see Barth et al., 1985, "The Murine T Cell Receptor Employs a Limited Repertoire of Expressed Vß Gene Segments", *Nature*, 316:517-523). According to this nomenclature, members of the same subfamily share the first digit and differ in second; therefore the

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Vß8.1, Vß8.2 and Vß8.3 are all members of the Vß8 subfamily. A similar system has been proposed for the human coding regions. In humans, the approximately 60 functional Vß coding regions have been grouped into at least 24 families (Toyonaga and Mak, 1988, *Ann. Rev. Immunol.*, 5:585; Wilson et al., 1988, *Immunol. Rev.*, 101:149; and Robinson, M.A., 1991, *J. Immunol.*, 146:4392).

2. T CELLS IN AUTOIMMUNITY

T cells play a pivotal role in the differentiation and regulation of effector mechanisms within the immune system (Paul et al., 1987, *Science*, 195:1293-1300). The co-recognition of antigen and major histocompatibility molecules by a T cell must be specific and precisely regulated, since improper immune regulation fosters autoimmunity. Several laboratories have studied diseases in which there appears to be improper immune regulation, such as autoimmunity and some forms of immunodeficiency, and have implicated T cells in the pathogenesis of such diseases.

Several undesirable immune responses are characterized by a clonal or oligoclonal expansion of a particular TCR composition as, for example, in conditions of malignancy which have resulted in a T cell leukemia or lymphoma. (See, e.g., Jack et al., 1990, *Am. J. Pathol.*, 136:17; Nitta, et al, 1990, *Science*, 249:672; and, Yoshino et al., 1991, *Int. J. Cancer*, 47:654). In T cell leukemias or lymphomas, the TCR acts as a unique tumor marker because it is stably rearranged and presented on the surface of the cell. A particular TCR composition is also implicated in the recipient of an organ graft whose T lymphocytes have TCRs reactive with the MHC antigens of the donor individual, as for instance the donor of a bone marrow graft.

Several groups have reported selective TCR V region expression in certain autoimmune situations. For instance, Grunwald et al., have noted a preferential expression of the Vα2.3 gene product in CD4+ T cells in the bronchoalveolar lavage when compared to peripheral blood lymphocytes of patients with Sarcoidosis (Grunwald, J. et al., 1992, *Eur J. Immunol.*, 22:129). In Kawasaki disease the preferential expansion of Vß2 and Vß8 T cells was noted at the onset of disease (Abe, J., et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066).

Rheumatoid arthritis (RA) has been extensively studied in this regard. Several investigators have noted preferential expansion of certain of T cell subsets, e.g., DerSimonian, et al., 1993, *J. Exp. Med.*, 177:1623 (preferential expansion of Vα12.1-bearing T cells in CD8+ peripheral blood T lymphocytes); Stamenkovic, et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:1179 (synovial membrane-infiltrating T cells grown in IL-2 were oligoclonal by Southern blot analysis); Paliard, et al., 1991, *Science*, 253:325 (hypothesizing that superantigen-activated Vß14 T cells, including autoreactive T cells, expand clonally and migrate to the synovial fluid of RA patients); Howell et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:10921 (noting particularly Vß3, 14, and 17 T cell V region gene usage in IL-2R+ cells from synovial fluid of RA patients); Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*,

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88:8534 (oligoclonal T cell V region gene usage in synovial fluid T cells of a single RA individual); International Patent Publication WO 90/06758 (implicating Vß3, 9, and 10 in RA).

Inflammatory bowel disease has also been extensively studied. Several groups have noted expanded T cell population or preferential TCR V region gene usage, e.g., Posnett et al., 1990, *J. Clin. Invest.*, 85:1770; Spencer et al., 1991, *J. Clin. Pathol.*, 44:915; Trejdosiewicz et al., 1991, *Clin. Exp. Immunol.*, 84:440; Van Kerckhove et al., 1992, *J. Exp. Med.*, 175:57. Still others have reported preferential TCR V gene usage in *Mycobacterium leprae* (van Shooten et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:11244; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188.

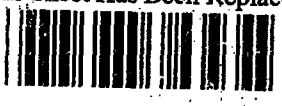
Multiple sclerosis (MS) is an autoimmune disease characterized by central nervous system mononuclear cell infiltration and demyelination. Although the pathogenesis of MS is unknown, both genetic and environmental factors have been implicated in the disease process. Major elements of the genetic predisposition include an association of disease with particular class II major histocompatibility complex (MHC) haplotypes (Terasaid et al., 1976, *Science*, 1933:1245-1247; Ho et al., 1982, *Immunogenetics*, 15:509-517: Spielman et al., 1982, *Epidemial. Rev.*, 4:45-65; Francis et al., 1986, *Lancet*, 1:211; Elian et al., 1987, *Disease Markers*, 5:89-99 ; Urban et al., 1988, *Cell*, 54:577-592; Vandenbark, et al., 1989, *Nature*, 341:541-544; Howell et al., 1989, *Science*, 246:668-670).

It has been shown that T cells isolated from the cerebral spinal fluid of patients suffering from MS utilize a limited set of TCR V region genes. The demonstration of *in vivo* activated myelin basic protein (MBP)-specific T cells in MS patients implicates MBP-reactive T cells in the pathogenesis of the disease (Wucherpfennig, K.W., et al., 1990, *Science*, 248:1016-1019). Studies using polymerase chain reaction (PCR) amplification of cDNA with TCR Vß primers indicated that MBP-reactive T cell lines use only a limited number of Vß coding regions (Wucherpfennig, K. W., et al., 1990, *supra* (Vß17, and to a lesser extent Vß12, are frequently used in the recognition of the immunodominant region of the human autoantigen MBP); Oksenberg J. R., et al., 1993, *Nature*, 362:68-70 (rearranged Vß5.2 and/or Vß5.3 genes were detected in the brains of patients with a certain HLA phenotype); Kotzin, B. L., 1991, *Proc. Natl. Acad. Sci. USA*, 88:9161-9165 (a bias for use of the ß chain variable region 5.2/5.3, and to a lesser extent Vß 6.1, seen among MBP-specific clones from patients with MS)). The results of some studies also imply that there is a limited TCR Vα gene expression in MS brain lesions (Oksenberg, J. R., et al., 1990, *Nature*, 345:344-346).

3. APPROACHES TO T CELL MEDIATED THERAPY

In the past, efforts to selectively alter the T cell population of an individual have focussed on vaccination with whole live or attenuated T lymphocytes (for a review of this approach see Cohen, I.R.,1986, *Immunol. Rev.*, 94:5-21) or with peptide vaccines based on selecting a specific peptide portion of the TCR V region. These approaches have been based on the current view that potentially pathogenic T cells preferentially utilize a limited set of V coding regions. The approach

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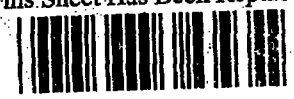
developed by Cohen uses live or attenuated T cells as a vaccine to treat or prevent, *inter alia*, experimental autoimmune encephalomyelitis (EAE). However, this approach is limited because it depends on the isolation if clones of disease-specific T cells: it is necessary to treat each patient with his own expanded cells, thus requiring "tailor-made" or individualized therapeutics. (See, Zhang, et al., 1993, *Science*, 261:1451).

Others have suggested the therapeutic use of TCR peptides based on TCR V region involvement (Offner, H., 1991, *Science*, 251:430 (treatment with a peptide based on the TCR Vß8 amino acid sequence reduced severity of disease and accelerated recovery in Lewis rats); Hashim, G.A., et al., 1992, *J. Immunol.*, 149:2803-2809; Offner, H. et al., 1992, *J. Immunol.*, 148:1706-1711; Offner, H., et al., 1991, *J. Immunol.*, 146:4165-417; *but see* Sun, D., 1992, *Cell. Immunol.*, 141:200-210). These peptides are generally short peptides, usually between 8-25 amino acids in length, and rarely more than 30 amino acids in length. For example a 21 amino acid peptide present on the TCR chain associated with EAE has been used to prevent EAE in Lewis rats (Vandenbark A.A. et al., 1989, *Nature*, 341:541-544; Vandenbark, A.A., International Patent Publication WO 91/01133; see also, Janeway, C.A., 1989, *Nature*, 341:482-483). Others have reported that even shorter peptides of 8 or 11 amino acids, corresponding to portions of the ß chain VDJ region or the Jα region proved effective as vaccines for either preventing or reducing the severity of EAE in a rat model (Howell, M.D. et al., 1989, *Science*, 246:668-671; Howell, M.D. et al., International Patent Publication WO 92/12996).

Peptides derived from TCR proteins have commonly been chosen as candidates for autoimmune therapeutics because of the ease of peptide synthesis and because no method has been previously available for the production of longer TCR V region proteins in useful quantities and having significant biological activity. More importantly, the major drawback of small synthetic peptides is that their use requires a prediction of the particular sequence that would be effective in modulating an immune response. Predictions must be made as to the antigenic or immunogenic properties of the proposed peptides, as for example their occurrence in the hypervariable or complementarity-determining region (CDR) of a V region. Additionally, the size of the proposed peptide derived from a particular TCR must be calculated to maintain the proper epitope structure to prime suppressor T cell activity or to elicit release of antibodies directed against the subpopulation of T cells bearing that particular TCR (to which the peptide corresponds). Such peptide design requires substantial time and experimentation; and in many instances where human therapies are concerned, the necessary experimentation may not be performed, for humane or ethical reasons.

In addition, in order to obtain a specific antigenic response by a regulatory T cell, the peptide must be bound to an individual's MHC molecules. These MHC antigens present on target cells have polymorphic regions wherein specific alleles are associated with particular individuals. For the most part, the individual will have two haplotypes, meaning that there will be two different

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alleles of a particular MHC antigen from the same locus. Not all peptides will have the capacity to bind to MHC antigens, nor will all MHC antigens bind to any single peptide, since there are multiple MHC antigens for each haplotype. Therefore, the suitability of such a peptide approach to T cell-mediated therapeutics must be determined on the haplotype of each individual, requiring tailoring of peptides to the individual patient.

4. PRODUCTION OF SOLUBLE T CELL ANTIGEN RECEPTOR

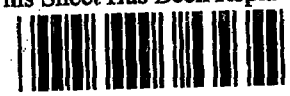
Production of large amounts of soluble TCR proteins by recombinant techniques has been problematic for several reasons. A major obstacle to overcome in the production of soluble T cell antigen receptor is the secretion in the absence of the transmembrane region (Traunecker, A. et al., 1989, *Immunol. Today*, 10:29-32). One investigator was able to show secretion of a full length monomeric ß chain of the murine TCR including the V, D, and J regions followed by a truncated form of the Cß region. The methodology took advantage of the homology between the carboxyl-terminal end of the immunoglobulin light chain and the sequence around Cys 241 in the TCR ß chain. The resulting construct utilized the chimeric gene encoding the k-chain leader peptide sequence from MPC-11 and a rearranged VDJ exon. The resulting construct was expressed in mammalian cells and was detectable in the cell supernatant. The method did not suggest, however, a means of obtaining a full length V region protein in the absence of extraneous protein sequences, since secretion was dependant on the presence of the truncated Cß exon (Gasgoigne, N., 1990, *J. Biol. Chem.*, 265(16):9296-9301).

Another group attempted to achieve the expression of a soluble form of the TCR ß chain in biochemically significant quantities by the expression of a recombinant TCR heterodimer anchored by a phosphatidyl inositol glycan linkage. The method produced small (0.5mg/harvest) amounts of a full TCR chain which necessitated the cleavage from the surface of the transfected cells by a phosphatidyl inositol-specific phospholipase C (Lin, A.Y., 1990, *Science*, 249:677-679).

Others have produced chimeric molecules by shuffling the variable and constant domains of the murine TCR with the constant region of an immunoglobulin κ light chain. The investigators were able to achieve small amounts of a secreted soluble form of a heterodimeric αß TCR (Gregoire, C., et al., 1991, *Proc. Natl. Acad. Sci.USA*, 88:8077-8081). Gregoire et al. show a murine chimera consisting of the Cα and Vα genes of the KB5-C2 joined to the C region of the κ light chain of the S105 monoclonal antibody. Also shown is a VßCßCκ chimera. Both are transfected into a mammalian B cell myeloma that does not express native immunoglobulin heavy or light chains. (See also, Weber, S., et al., 1992, *Nature*, 356:793-795).

Novotny et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8646-8650, demonstrate a method for producing soluble TCRs with antigen-combining properties. A synthetic oligo linker was cloned onto the C terminus of the Vß and the N terminus of the Vα region resulting in a single-chain VßVα. The resultant expression vector encodes the pelB leader followed immediately by in frame

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Vß, the linker, and the Vα segments. The expression of this construct is under the control of the lacZ promoter and is therefore IPTG-inducible.

Recently, one group has shown the secretion of a small amount of a T cell receptor V region fused to a carboxyl-terminal poyhistidine "tail". The plasmids contain the pelB leader and are under the control of a chemically inducible promoter (Ward, E.S., 1991, *Scand. J. Immunol.*, 34:215-220). The work fails, however, to describe how to obtain a T cell antigen receptor V region protein in the absence of a fusion partner, or how to obtain commercially useful amounts of the receptor V region.

There is a great need, therefore, for agents and pharmaceutical compositions which have the properties of specificity for the targeted autoimmune response, predictability in their selection, and convenience and reproducibility of preparation.

SUMMARY OF THE INVENTION

The present invention addresses the above-mentioned problems by providing for the first time the successful production of a universal agent for treating a given T cell-mediated disease in which T cell subsets are implicated. In particular, the invention provides useful quantities of full length TCR V region proteins that may be naturally processed, providing for multiple active fragments upon *in vivo* processing, and presented by antigen-presenting cells of each individual, thereby eliminating the need for "tailor-made" therapeutic agents. Also provided is a class of recombinant expression vectors for the production of TCR V region proteins in bacteria. The recombinant TCR V region proteins of the instant invention are useful in the treatment, prevention or suppression of an immune-related disorder. In another embodiment, the TCR molecules can be combined in an admixture with a pharmaceutically acceptable carrier for use as a therapeutic composition. In another embodiment, the claimed TCR V region proteins are useful to generate anti-TCR antibodies. Alternatively, the TCR V region proteins can be used diagnostically to characterize a particular immune-related disorder. The TCR V region proteins of the instant invention can be used as diagnostic probes based their ability to bind with autoantibodies. It is an advantage of the present invention that the T cell antigen receptor V region proteins are expressed in the absence of fusion proteins or affinity tails. It is a further advantage of the present invention that the TCR V region proteins are produced in commercially acceptable amounts that enable the preparation of compositions which are therapeutically effective in treating T cell-mediated autoimmune disease.

The invention further provides for an expression vector for the production of a pure full length TCR V region molecule. The expression vectors of the invention include as an essential element a coding region for a T cell antigen receptor V region. The TCR coding region of the invention is any nucleic acid molecule encoding a full length TCR V region protein. In a preferred embodiment, the TCR coding region includes the structural coding sequence for the Vß5.3 region.

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The expression vectors described herein provide a means for the inducible expression of TCR V region proteins. In preferred embodiments, the T cell antigen receptor V region coding sequence is under the transcriptional control of an inducible bacterial promoter. According to the present invention, the inducible bacterial promoter is subject to either chemical or thermal regulation. Most preferably, the inducible bacterial promoter is subject to thermal induction.

In one embodiment, the expression vector of the instant invention includes a regulatory gene coding for a protein capable of binding to the promoter and thereby repressing transcription from the inducible promoter. In a preferred embodiment, the promoter is derived from the *E. coli lac* operon and the regulatory gene comprises a functional mutation of the *E. coli lacI* repressor gene. This mutant *lacI* gene (*lacI*ts) codes for a temperature-sensitive repressor protein, and utilizing this *lacI*ts gene permits thermal induction of the promoter. In a preferred embodiment, the inducible bacterial promoter is a *trc* promoter, and the thermal induction is provided by the *lacI*ts regulatory gene.

In one embodiment, the regulatory gene is located on a cotransformed vector. In another embodiment, the regulatory gene is located in the bacterial host cell chromosome. In a preferred embodiment, the regulatory gene is located in the recombinant expression vector. In a further preferred embodiment, the transcription direction of the mutant *lacI* gene is in the same orientation as the T cell antigen receptor structural gene to be expressed.

The recombinant expression vectors of the present invention may advantageously include a coding sequence for a leader or signal peptide. Such a leader sequence, fused in frame and 5' to the TCR Vregion coding region to be expressed, directs the synthesis of a TCR V region protein having an N-terminal leader peptide which in turn directs secretion of the recombinant protein across the host cell membrane. Other embodiments of the present invention do not utilize a leader sequence, and the desired V region product is isolated from the cells, e.g., by urea extraction.

In a preferred embodiment, the recombinant expression vectors of the present invention comprise, in the 5' to 3' direction: a *trc* promoter, a ribosome binding site, an optional leader sequence, a coding region for a full length T cell antigen receptor V region, a stop codon, one or more transcription terminators, a *lacI*ts regulatory gene such that the transcription direction is the same as that of the coding region for the T cell antigen receptor V region, a bacterial origin of replication, and a tetracycline resistance gene.

This invention also provides methods for producing TCR V region proteins at significantly increased levels than previously reported.

The present invention provides compositions and methods for treating a patient having an immune-related disorder. Such treatment comprises administering to a subject in need of such treatment, a composition in accordance with the present invention, in an amount and for a period of time suitable to regulate said immune disorder. It is an advantage of the present invention that the compositions of the invention allow for treatment of an immune related disorder without the need to

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select a particular peptide fragment and without tailoring the particular composition to the particular individual to be treated.

The invention also relates to the use of a full length T cell antigen receptor V region protein in therapy for an autoimmune disease or disorder. In a specific embodiment, the invention relates to the use of a full length Vß5.3 protein in therapy. In another embodiment, the invention provides for the use of a full length T cell antigen receptor V region protein in the manufacture of a medicament for treating an autoimmune disease or disorder. Therefore, in one embodiment, the invention enables the use of a full length Vß5.3 protein in the manufacture of a medicament for the treatment of an immune related disease or disorder such as MS.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. The complete nucleotide sequence [SEQ ID NO. 25 and SEQ ID NO. 26] and amino acid sequence [SEQ ID NO. 27] of the full length Vß5.3 protein of the invention.

Figure 2. Plasmid map of pKTB-Vß5.3. This plasmid contains Ptrc, the *trc* promoter; g10, the g10 leader gene segment from bacteriophage T7; SD1, ribosome binding site 1; MC, a mini-cistron encoding ribosome binding site 2 (SD2) AAG GAG (see SEQ ID NO. 28 for complete mini-cistron nucleotide sequence) and the peptide Met Tyr Arg Leu Asn Lys Glu Glu (SEQ ID NO. 29); STII, the STII leader sequence; Vß5.3, the Vß5.3 coding region; T1T2, transcription terminators of the *rrnB* operon; the *lacI*ts gene encoding the temperature-sensitive *lac* repressor; Ori, the origin of replication and Tet^r, the tetracycline resistance gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes recombinant expression vectors and methods for the efficient production of large quantities of a T cell antigen receptor V region protein and the use thereof, including the diagnosis, treatment, prevention or suppression of immune-related diseases or disorders.

A series of vectors has been developed for the production of enhanced levels of a full length TCR V region protein. Additionally, a method is provided for producing commercially useful amounts of a full length recombinant TCR V region protein using an expression vector of the instant invention. The methods and expression vectors of the instant invention are unique because they provide for the production of a full length T cell antigen receptor V region in the absence of extraneous protein sequences or fusion partners. As detailed in the Background of the Invention, *supra*, T cell antigen receptor V regions have, in the past, been produced only as part of a larger protein, and at very low levels. These extraneous proteins may cause undesirable immunogenicity when used *in vivo*, and they are frequently cited by regulatory authorities as impermissible impurities. Previously purified V region proteins included other regions of the TCR, such as the C region, fused to the V region. Others constructed expression systems that provided for a TCR V region as a chimeric protein, e.g., a V region fused to a portion of an immunoglobulin molecule.

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The expression vectors and methods of the instant invention further provide for enhanced levels of expression of a T cell antigen receptor V region protein. A primary consideration in the production of a protein that has commercial utility, for example, in research, diagnostics and therapeutics, is the manufacture of commercially useful amounts of a protein. The present invention therefore provides for the production of enhanced levels of TCR V region proteins. The present invention makes possible the production of V region protein in large amounts, e.g., from 1 and 500 mg/liter of bacterial culture, or more. Preferably, the production level of the TCR V region protein is at least 100 mg/liter. In a most preferred embodiment, the instant invention provides for the production of at least 300 mg of Vβ protein per liter of bacterial cell culture.

The methods and expression vectors of the instant invention provide for the production of a TCR V region protein in the absence of extraneous protein sequences. The invention therefore eliminates several steps associated with obtaining a purified TCR V region protein if prepared according to the prior art. In one embodiment, the present invention provides for increased expression of V region proteins by thermal induction, which avoids the necessity of eliminating chemical inducers, such as IPTG.

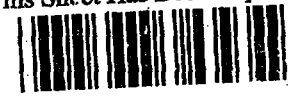
Because TCR V region proteins are produced according to the invention in the absence of extraneous proteins, the invention provides for simplified production of relatively pure product. The invention eliminates additional processing steps, such as the introduction of enzymes or chemical reagents into the system to obtain a protein free of any fused non-TCR peptide segments or affinity tails. Not only are such manipulations likely to reduce the amount of V region product recovered, they also introduce elements which must be eliminated, i.e., by additional purification steps.

1. T CELL ANTIGEN RECEPTOR V REGION

The T cell antigen receptor V region protein of the instant invention is any full length T cell antigen receptor V region. A full length TCR V region protein is encoded by a coding region for a T cell antigen receptor V region. The TCRV region coding region is a nucleotide sequence encoding a full length T cell antigen receptor V region protein. Within the scope of the present invention, V region includes the variable region of the T cell antigen receptor. In its broadest aspects, the teachings of the present invention may be applied to obtain enhanced expression of TCR proteins including the V region and, additionally, all or part of the D and/or J regions.

TCR V region protein is produced in the absence of extraneous protein sequences, such as, for instance, other TCR regions, such as the constant region, the transmembrane region or the cytoplasmic region. Further, the TCR V region protein of the invention is produced in the absence of non-TCR fusion partners, such as affinity tails. Further, the TCR V region protein of the invention is not produced as a part of a larger chimeric protein including portions of other protein molecules, such as an immunoglobulin protein.

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Recognizing that the DNA sequence encoding one of the complementarity-determining regions (CDRs) of a T cell receptor's V region may actually span the known juncture between the V and D coding regions (as in the case of the CDR3 of Vβ 5.3), as used herein, a "full length" V region coding region may include the entire coding sequence of such a CDR that spans the V-D juncture, the portion of such a CDR that only resides in the known V region, or none of the sequence coding for such a CDR. Furthermore, the full length T cell antigen receptor V region protein of the present invention can be any of the T cell antigen receptor V regions and is not limited to only Vβ regions (i.e., V regions of the TCR β-chain). Therefore, the T cell antigen receptor V region protein can be a variable region of the α subunit, the β subunit, the δ subunit, or the γ subunit.

In a preferred embodiment, the TCR V region protein of the instant invention is any T cell antigen receptor V region for which a full length coding sequence is available. Methods of obtaining the full length coding sequences of the instant invention are well known in the art. Human V region domain DNA sequences can be isolated in accordance with well known procedures from a variety of human cells. Preferably, the cells are peripheral blood T lymphocytes.

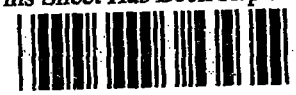
The DNA sequences of the present invention may be prepared in various ways. It is preferred that the DNA sequences of the T cell antigen receptor V regions be prepared by polymerase chain reaction (PCR) using DNA sequences coding for the intact TCR as templates. A human Vβ region can be prepared, for instance, via amplification of cDNA prepared from human peripheral blood lymphocytes stimulated by an anti-CD3 antibody, following the procedure of Choi et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:8941-8945.

In one aspect of the present invention, the coding region for a full length T cell antigen receptor V region is obtained from any clone containing the full length sequence. For example, several TCR V region sequences and cDNA clones containing the full length coding regions are described in Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602.

Preferred TCR V region proteins of the instant invention are those implicated in an immune-related disease or disorder, particularly, e.g., Vβ 8.1 (Kawasaki disease; see, Abe et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066), Vβ 6.1 (leprosy; see, Wang, et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188), Vβ5.1 (Lyme disease; see, Lahesma et al., 1993, *J. Immunol.*, 150:4125), Vβ5.2, or Vβ5.3, or Vβ6.1 (multiple sclerosis; see Kotzin, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:9161), Vβ2.1, or Vβ3.1 (rheumatoid arthritis; see Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8534). (See also, Hafler, D.A. et al., 1988, *J. Exp. Med.*, 167:1313; and Mantgazza R. et. al., 1990, *Autoimmunity*, 3:431).

The TCR expressed by a T cell clone in an autoimmune disease or a T cell clone responding to a particular autoantigen can be identified using TCR-specific antibodies, for example polyclonal, monoclonal or chimeric antibodies that are specific for a TCR variable segment, in conjunction with an antibody labelling method, including fluorescence microscopy, flow cytometry,

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immunocytochemistry, or other techniques known in the art. Such antibodies have been described for a number of TCR α and/or β chain V regions (see, for example, International Patent Publication WO 90/06758).

Alternatively, the DNA or mRNA of the T cell clone can be probed directly, or after amplification by PCR (Synha et al., 1988, *Science*, 239:1026; Saiki et al.,1986, *Nature*, 324:163), and specific hybridization with nucleic acid probes for the various TCR gene families, using hybridization methods well known in the art. The TCR sequence, or a part thereof, can then be obtained directly from the amplified, rearranged DNA or mRNA.

Expression of a particular TCR can also be identified by determining the nucleic acid sequence encoding at least part of the TCR, for example, after cloning the TCR V gene, or by determining the amino acid sequence of at least part of a TCR protein. It will be apparent that any of the above-mentioned approaches, or additional approaches known to one of skill in the art, will result in the identification of the TCR expressed on a T cell or clone or line of T cells.

Examples of molecular approaches used to correlate TCR gene expression with disease include:

(1) producing and analyzing cDNA libraries obtained from the disease-related T cells obtained from one or more subjects having the disease, to determine the presence of frequently used or 'dominant' TCR genes;

(2) Southern analysis of disease samples to determine whether specific genetic polymorphism (e.g., RFLPs) or oligoclonal TCR rearrangements exist;

(3) analysis of disease samples by cDNA synthesis, PCR amplification, and slot blot hybridization procedures; and

(4) *in situ* nucleic acid hybridization of TCR probes to T cells without prior culture of these cells.

Preferred TCR V region proteins include Vβ8.1, Vβ5.2, or Vβ5.3. Double-stranded cDNA encoding Vβ5.3 and the amino acid sequence for Vβ5.3 are shown in Figure 1. (See, SEQ ID NOS. 25, 26 and 27.)

A T cell antigen receptor V region clone can be introduced into the expression vectors of the instant invention by methods known to those skilled in the art. For example, from a cDNA clone, the V region is PCR synthesized incorporating an initiating ATG at the 5' terminus. Any convenient restriction site can be synthesized which incorporates an initiating codon and reconstitutes the 5' coding region of the V region. In a preferred embodiment, the restriction site is an *Nco*I site. A stop codon and an appropriate restriction site are engineered at the 3' end of the V region coding sequence so that it can be subcloned into the vector.

It should be understood that the methodology of the present invention can be used to prepare full length TCR V regions derived from animal species other than human.

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2. EXPRESSION VECTORS

A recombinant expression vector within the scope of the present invention is a plasmid which contains exogenous DNA in the form of a nucleic acid coding TCR V region to be expressed. The recombinant expression vector of the present invention is introduced into a bacterial host cell for example by transformation, and transformants are isolated and cloned which express the desired V region protein.

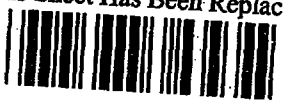
The present invention concerns expression vectors containing the DNA sequence coding for the full length TCR V region protein. Preferably, the TCR V region proteins are those T cell antigen receptor proteins specifically described herein. An expression vector within the scope of the present invention is a plasmid which contains the TCR V region gene to be expressed under the transcriptional and translational control of regulatory elements, such as for instance the promoter, ribosomal binding site and transcription terminator. In addition it contains an origin of replication for stable replication in bacteria. It also preferably includes a gene coding for resistance to an antibiotic to facilitate selection of plasmid-bearing transformant cells. Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell.

Expression vectors useful in the present invention are often in the form of plasmids. Plasmids refer to circular double-stranded DNA sequences which are not integrated in the chromosome. The expression vectors of the present invention may also include other DNA sequences, for example leader or signal sequences which provide for targeting or stability of the expression product, and/or regulatory sequences which allow modulation of gene expression, for example in response to a shift in temperature or a change in composition of the growth medium.

The expression vectors contain one or more control sequences operably linked to the DNA sequences coding for the TCR V region. The term "operably linked" within the scope of the present invention means that one nucleic acid sequence ,which has a regulatory function for gene expression or encodes a functional protein domain, is covalently linked, either 5' or 3', to a second nucleic acid sequence encoding a protein, so that the regulatory function is exerted over the second nucleic acid sequence or the encoded functional domain will be fused in frame to the protein encoded by the second nucleic acid. Such regulatory functions for gene expression typically include initiation of transcription, termination of transcription, and mRNA transcript processing. Nucleic acid sequences that exert such regulatory functions over gene expression include promoters of transcription, transcription terminators, and mRNA processing signals. Functional domains that may be "operably linked" to another protein include, for example, leader or signal peptides which permit transport of proteins across cell membranes.

In one embodiment, the promoter may be any inducible promoter. In a preferred embodiment, the promoter is one that is regulated by the presence or absence of certain media components. One skilled in the art will recognize that several bacterial promoters subject to the

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control of the presence or absence of certain media components can be used in connection with the expression vectors of the instant invention. It is preferred that the promoter be a strong bacterial promoter such as the *lac* promoter, the *tac* promoter, and the *trc* promoter. Therefore, in one embodiment, a promoter for a TCR V region coding sequence in a vector of this invention is subject to regulation by the presence or absence of isopropyl-ß-D-thiogalactopyranoside (IPTG), a gratuitous chemical inducer molecule of the *lac*, *tac*, and *trc* promoters. In preferred aspects, the IPTG-inducible promoter is the *lac* promoter or the *tac* promoter. In a more preferred embodiment, the IPTG-inducible promoter is the *trc* promoter (Brosius et al., 1985, *J. Biol. Chem.*, 260:3539-3541) which is similar to the *tac* promotor derived by fusing the -10 region of the *lac* UV5 promoter with the -35 region of the tryptophan operon (de Boer et al., 1983, *Proc. Natl. Acad. Sci. USA*, 80:21-25; Amann et al., 1983, *Gene*, 25:167-178.) The *trc* promoter is identical to the *tac* promoter with the exception of a single base pair change in the promoter (Brosius et al., (1985) *supra*).

In a further embodiment, the expression vector of the instant invention is modified to allow for the secreted expression of the full length TCR V region protein of the invention by a temperature shift. According to this embodiment, the expression vector allows for the expression of TCR V region protein in the absence of chemical inducers, which obviates the need to use such elements as IPTG and bacterial host strains containing the *lacI* or the *lacI*^{q} genes. Preferred embodiments of the expression vectors of the instant invention include a regulatory gene which allows for the thermal induction of protein expression. The regulatory gene sequence most preferably is the *lacI*ts gene, which encodes a temperature-sensitive *lac* repressor.

In a further preferred embodiment, the *lacI*ts gene is located proximal to and downstream of the transcription terminator, and its transcription direction is the same as that of the V region coding sequence.

In addition, the expression plasmids of this invention preferably contain one or more selectable genetic marker(s). Preferably, the selectable genetic marker confers resistance to a antibiotic, such as ampicillin, kanamycin or tetracycline. Most preferably, the selectable marker is the tetracycline resistance gene.

Additionally, the vector includes a bacterial origin of replication. In a preferred embodiment, the origin of replication is derived from pBR322. The expression vector also contains a ribosome binding site and the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118.)

In a preferred embodiment, an expression vector of the instant invention contains as essential elements in the 5' to 3' direction: a DNA sequence which contains a promoter and an operator; a DNA sequence which contains a ribosomal binding site for rendering the mRNA of the desired gene capable of binding to ribosomes within the host cell; an ATG initiation codon or a DNA sequence which is converted into an ATG initiation codon upon insertion of the desired gene

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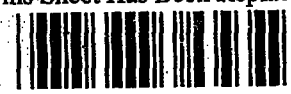
into the vector; a restriction site for the insertion of the desired gene in frame with the ATG initiation codon or a multiple cloning site containing several restriction sites for the insertion of the appropriate gene or coding region to be expressed; a nucleic acid sequence encoding the T cell antigen receptor V region to be expressed; and a transcription terminator which serves to efficiently terminate transcription. The vector also contains a DNA sequence which contains an origin of replication from a bacterial plasmid capable of autonomous replication in the host cell and a DNA sequence which contains a gene associated with a selectable or identifiable phenotypic trait which is manifested when the vector is present in the host. The expression vector may optionally include a signal or leader coding sequence operably linked, i.e., fused in frame, to the 5' end of the nucleic acid sequence encoding the TCR V region so that translation of the leader sequence-TCR V region sequence results in a leader peptide-TCR V region fusion protein in which the leader peptide at the amino terminus of the fusion protein directs secretion of the TCR V region protein across a host cell membrane. In certain preferred embodiments, the expression vector contains a regulatory gene which allows for the expression of the secreted protein by a temperature shift.

In a more preferred embodiment, the expression vector contains the following essential elements in 5' to 3' direction: The *trc* promoter which is composed of the -10 region of the *lac* UV5 promoter fused with the -35 region of the tryptophan operon (de Boer et al., *supra*; Amann et al. (1983) *supra*); a strong ribosome binding site; the TCR V region coding region to be expressed; the two transcription terminators from the *rrnB* ribosomal RNA operon; the *lacI*ts gene coding for the temperature-sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. An example of such a preferred embodiment, the expression vector pKBi-Vβ5.3 was deposited under the Budapest Treaty with the American Type Culture Collection (ATCC) under accession no. 69739 on January 6, 1995.

Expression vectors of the invention may contain a leader sequence encoding a signal peptide operably linked 5' of the TCR V region coding region. On expression, amino-terminal signal peptide will direct secretion of the TCR V region protein across the host cell membrane, into the periplasm or the culture medium. One skilled in the art will recognize that many leader sequences may be used in connection with the expression vector of the instant invention. One suitable leader sequence is the pelB signal sequence from *E. carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383). Another suitable leader sequence is the heat stable enterotoxin II (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732).

In one embodiment, the expression system contains the STII leader sequence, and the coding region for a V region is PCR synthesized to contain an appropriate restriction site at the 3' end and an engineered *MluI* site at the 5' end so that it can conveniently link to the *MluI* site incorporated into the 3' end of the STII leader. Therefore, in a further preferred embodiment, the expression vector comprises in the 5' to the 3' direction: the *trc* promoter which is composed of the -10 region of the

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lac UV5 promoter fused with the -35 region of the tryptophan operon; a strong ribosome binding site; the STII leader; the coding region for the T cell antigen receptor V region to be expressed; the transcription terminators from the *rrnB* operon; the *lacI*ts gene coding for the temperature sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. In a most preferred embodiment, the expression vector is pKB-Vβ5.3.

The expression vectors of the present invention may be constructed using standard techniques known in the art. (See, e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

The present invention additionally concerns host cells containing an expression vector which contains a DNA sequence coding for a full length TCR V region. Additionally, preferred host cells contain an expression vector comprising one or more control DNA sequences capable of directing the replication and or the expression of a DNA sequence coding for a full length T cell antigen receptor V region protein. Suitable host cells include prokaryotic host cells; and in preferred aspects, the prokaryotic host cell is a bacterial host cell, most preferably an *E. coli* cell. One skilled in the art will understand from the teaching of the instant application that the particular expression vector used will influence the choice of host strains selected for the production of the full length TCR V region proteins. For instance, in the expression vectors subject to chemical induction within the scope of the present invention, bacterial host strains which harbor the *lacI* or *lacI*^{q} gene, which regulate the *trc* promoter, are especially useful. In expression vectors within the scope of the present invention that contain a regulatory gene such as the *lacI*ts gene, which provides for a temperature-sensitive repressor, bacterial host strains that do not harbor the wild type *lacI* or *lacI*^{q} genes are preferred. A particularly preferred host cell is LJ24.

In certain embodiments, the expression vector of the instant invention does not contain a leader sequence. In certain preferred embodiments the bacterial host strain is *E. coli* BL21, or more preferably a protease deficient *E. coli* strain, such as SG21163, SG22094, or SG21173.

The expression vectors of the present invention may be introduced into the host cells by various methods known in the art which may vary depending on the type of cellular host. For example, calcium chloride treatment or electroporation are commonly utilized for prokaryotic cells. (See, generally, Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

Once the expression vector has been introduced into an appropriate host cell, the host cell may be cultured under conditions permitting expression of large amounts of TCR V region.

Host cells containing an expression vector which contains a DNA sequence coding for a TCR V region protein may be identified by any appropriate means. In the following examples, for instance, production of V region protein is detected immunologically using an immunoassay. Alternatively, the host cell containing the expression vector of the instant invention may be

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identified by assessing the level of transcription of TCR V region DNA by measuring TCR-specific V region mRNA transcripts in the host cell (e.g., by Northern blot analysis). In yet another method, the host cell containing the expression vectors of the instant invention can be identified by DNA-DNA hybridization using probes complementary to the TCR V region coding sequence of the vector.

The DNA sequences of the present invention, expression vectors or DNA molecules may be determined by various methods known in the art. For example the dideoxy chain termination method as described in Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA.*, 74:5463-5467.

Following the examples provided herein and utilizing one or more of the detection methods described above, a person skilled in the art may, without undue experimentation, readily select the combinations of expression vectors and host cells that provide optimal expression of TCR V region proteins. By combining a particular host strain with a particular expression vector of this invention, it is possible to obtain relatively high level expression of full length T cell receptor protein. Preferably, the level of expression of full length TCR V region protein is at least 300 mg per liter of culture. Such levels of expression can be obtained, for example, by using a preferred embodiment in which *E. coli* SG21173 is the bacterial host strain and pKBi-Vβ5.3 is the expression vector (see examples, *infra*). Such combinations of host and expression vectors of this invention permit the production of significant quantities of full length T cell receptor protein at a lower cost than previously available.

Once expressed, the proteins of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity chromatography, column chromatography, gel electrophoresis and the like. (See. generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982).)

In a preferred embodiment, the T cell antigen receptor V region is soluble. By this it is meant that the T cell antigen receptor V region is soluble in an aqueous system. A "soluble" protein is generally characterized by its failure to sediment at room temperature under high G-force centrifugation (100,000 x g) out of an aqueous buffer. Solubility is particularly important in providing simplicity in purification of the TCR V region protein from the producing cell. Aqueous buffers used in protein chemistry typically have a buffering compound for maintaining a particular pH (typically within a physiological range of about pH 5-9), provide a particular ionic strength (typically between about 10 mM and about 500 mM), and often include a protease inhibitor and a mild detergent. Examples of standard buffers are phosphate buffered saline, Tris-buffered saline or any of a number of buffers used in protein isolation. (See, generally, *Methods in Enzymology*; Mahler and Cordes Biological Chemistry (2d Ed.), Harper and Row, New York (1966).)

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3. USE OF T CELL ANTIGEN RECEPTOR V REGION PROTEINS

The present invention provides for the first time the means for efficiently and economically producing diagnostically, therapeutically and investigationally useful quantities of a TCR V region protein that is not a fusion protein or linked to an extraneous sequence, such as an affinity tail.

A. Investigational use and diagnostic use

The TCR V region proteins of the present invention are useful in structural and functional studies on the T cell antigen receptor (TCR). Besides serving as substrates or binding domains for specific forms of TCRs, these proteins may serve as tools for conformational studies, e.g., by approximating the native conformation of various portions of the T cell receptor.

The T cell V region proteins can also be used as diagnostic probes. In one specific embodiment, the TCR V region proteins of the present invention may be used in determining the amount or the presence of a certain TCR V region subfamily in a biological sample. One such assay is described in Rittershaus C.W., International Patent Publication WO92/08981, entitled "Therapeutic and Diagnostic Methods Using Total Leukocyte Surface Antigens". As such, the present invention provides methods for diagnosing an immune-related disease, such as multiple sclerosis (MS), by detecting the specific subset of T cells associated with the disease.

In another embodiment, the present invention can be used in the generation of significant quantities of T cell antigen receptor V region proteins for use in antibody production. The antibodies can be prepared by using any of a number of techniques well known in the art. For producing a monoclonal antibody (MAb), any method which provides for the production of antibody molecules by continuous cell lines in culture may be used. These methods include, but are not limited to, the hybridoma techniques originally described by Kohler and Milstein, 1975, *Nature*, 256:495-497); the more recent human B cell hybridoma technique (Kozbor et al., 1983, *Immunology Today*, 4:72); the EBV technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)); and trauma techniques. For a review of antibody production methods see: Hartlaw, E., et al., Antibodies: A laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

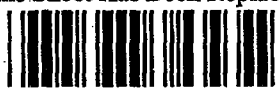
The TCR Vregion proteins of the invention can be used as diagnostic probes based on their ability to react with autoantibodies present in a biological sample.

The present invention also contemplates labeled derivatives of the TCR V region proteins which can be detected by appropriate monitors, such as scintillation and Geiger counters, or radiographic film. Such derivatives include, for example ^{125}I, ^{131}I, ^{14}C, ^{35}S, ^{3}H, ^{112}In, ^{99}M, Tc and the like, for *in vitro* and *in vivo* diagnostic purposes.

B. Therapeutic use

As mentioned above, the present invention is useful in the therapy of immune-related disease. The term "immune-related disease" as used herein refers to a disease in which the immune

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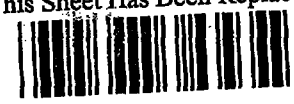
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system is involved in the pathogenesis of the disease, or in which appropriate stimulation of the immune system can result in protection from the disease. Relevant diseases include, but are not limited to, autoimmune diseases, neoplastic diseases, infectious diseases, hypersensitivity, transplantation, graft-versus-host disease, and degenerative nervous system diseases. Autoimmune diseases include, but are not limited to, arthritis, such as rheumatoid arthritis, type I diabetes, juvenile diabetes, multiple sclerosis, autoimmune thyroiditis (Hashimoto's thyroiditis), myasthenia gravis, systemic lupus erythematosus (SLE), Sjögren's syndrome, Graves disease, Addison's disease, Goodpasture's syndrome, scleroderma, dermatomyositis, myxedema, polymyositis, pernicious anemia, inflammatory bowel disease including Crohn's disease and autoimmune atrophic gastritis, and autoimmune hemolytic anemia. Neoplastic diseases include, but are not limited to, lymphoproliferative diseases such as leukemias, lymphomas, Non-Hodgkin's lymphoma, and Hodgkin's lymphoma, and cancers such as cancer of the breast, colon, lung, liver, pancreas, melanoma, renal carcinoma, etc. Infectious diseases include but are not limited to viral infections caused by viruses such as HIV, HSV, EBV, CMB, Influenza, Hepatitis A, B, or C; fungal infections such as those caused by the yeast genus *Candida*; parasitic infections such as those caused by schistosomes, filaria, nematodes, trichinosis or protozoa such as trypanosomes causing sleeping sickness, plasmodium causing malaria or leishmania causing leishmaniasis; and bacterial infections such as those caused by mycobacterium, corynebacterium, or staphylococcus. Hypersensitivity diseases include but are not limited to Type I hypersensitivities such as contact with allergens that lead to allergies, Type II hypersensitivities such as those present in Goodpasture's syndrome, and myasthenia gravis.

Therapeutic use of the TCR V region proteins of the invention is premised upon the correlation between a specific immune-related disease and the preferential expression of a particular T cell antigen receptor V region protein or the expanded usage of a particular sequence encoding a TCR V region protein. Though the present invention should not be limited by any particular scientific theory, the TCR V regions are useful in part because they may be used to regulate the immune response in an individual. Specifically, where the presence or abnormally high expression of a particular V region correlates with particular immune-related disorder, one can treat the individual by neutralizing the T cells carrying that particular V region protein. Further, introduction of the TCR V region protein may affect immunosuppressive functions by stimulating regulatory T cells which will specifically interact with T cells bearing a targeted V region. It is also likely that the TCR V region protein may elicit an antibody response that will specifically interact with the targeted V region on T cells and in turn modulate the immune response.

The full length T cell antigen receptor V region proteins of the instant invention provide distinct advantages in the treatment of an immune related disease. Prior to the instant invention, therapeutic options for treatment of immune related disease with specific T cell involvement

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included: (a) no treatment with possible spontaneous resolution; (b) treatment with peptides corresponding to distinct segments of the T cell antigen receptor V region; (c) nonspecific treatments such as steroids or non-steroidal anti-inflammatory agents. However, none of these afforded the particular advantage of the present invention. The therapeutic methods of the instant invention have the distinct advantage over the prior art methods of treating an immune related disease in that the methods of the present invention target only the particular T cell subset expressing a particular TCR V region and can be used across a diverse range of affected individuals, since the invention provides for the elimination of the need to select a particular peptide based on the MHC haplotype of a particular individual. The method of the present invention more closely approaches the goal of modulating only disease-related T cells while sparing or not affecting other T cells in the subject. The invention therefore achieves a greater specificity of therapy across a wider population.

In a preferred embodiment, a full length T cell antigen receptor V region protein may be used to down-regulate or eliminate a subset of T cells that exhibit a TCR incorporating the same V region. The T cell antigen receptor V region protein can be used in a therapeutic situation to modulate the activity of those T cells bearing the targeted V region or to induce unresponsiveness in the actively responding population of T cells bearing the targeted V region. In this situation the T cell antigen receptor is used as a therapeutic composition in a subject.

Specific T cell antigen receptor V region proteins associated with a given disease are identified using any of a variety of techniques well known in the art. A genetic approach using patients known to have myasthenia gravis or multiple sclerosis was described by Oksenberg, J.R., et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:988-992.

The term treatment in the instant invention is meant to include the situations of "prevention," "suppression" or "treatment" of the disease. "Prevention" involves administration of the protective composition prior to the induction of the disease. Thus, for example, in the animal model of experimental autoimmune encephalomyelitis, EAE, successful administration of a protective composition prior to injection of the encephalitogen that induces the disease resulted in "prevention" of the disease.

"Suppression" involves administration of the composition after the inductive event but prior to the clinical appearance of the disease. Again, using the EAE example, successful administration of a protective composition after injection of the encephalitogen, but prior to the appearance of neurological symptoms, resulted in "suppression" of the disease.

"Treatment" involves administration of the protective composition after the appearance of the disease. In the EAE example, successful administration of a protective composition after injection of encephalitogen and after clinical signs had developed resulted in "treatment" of the disease.

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For measuring preventative, suppressive or therapeutic benefit of the TCR V region protein of the present invention, certain clinical outcomes are measured. In particular, alteration of proliferative response of lymphocytes *in vitro* can be measured. Assays for measuring the therapeutic benefit of the particular V region protein are known in the art.

Animal model systems which can be used to screen the effectiveness of the proteins in protecting against or treating the disease are available. Systemic lupus erythematosus (SLE) is tested in susceptible mice as disclosed by Knight et al., 1978, *J.Exp. Med.*, 147:1653 and Reinersten et al., 1978, 299:515. Myasthenia gravis (MG) is tested in SJL/J female mice by inducing the disease with soluble AChR protein from another species as described in Lindstrom et al., 1988, *Adv. Immunol.*, 42:233-284. Arthritis is induced in a susceptible strain of mice by injection of Type II collagen as described by Stuart et al., 1984, *Ann. Rev. Immunol.*, 42:233-284. Adjuvant arthritis is induced in susceptible rats by injection of Mycobacterial heat shock protein as described by Van Eden et al., 1988, *Nature*, 331:171-173. Thyroiditis is induced in mice by administration of thyroglobulin as described by Maron et al., 1980, 152:1115-1120. Insulin-dependant diabetes mellitus (IDDM) occurs naturally or can be induced in certain strains of mice such as those described by Kanasawa, et al., (1984). EAE in mice and rats serves as a model for MS in humans. In this model, the demyelinating disease is induced by administration of myelin basic protein (MBP) as described by Paterson, P.Y., Textbook of Immunopathology, (Mischer et al., eds.), Grune and Stratton, New York, pp. 179-213 (1986); McFarlin, D.E. et al., 1973, *Science*, 179:478-480: and Satoh, J., et al., 1987, *J.Immunol.*, 138:179-184.

Of course, the identical proteins may not be effective in humans since they may not correspond to an appropriate site of the disease-associated human TCR. It is to be understood that modifications of the protein sequence delineated in a particular animal model may be required in order to treat subjects belonging to other species, including humans.

4. PHARMACEUTICAL COMPOSITIONS CONTAINING THE T CELL ANTIGEN RECEPTOR V REGION

The proteins and compositions of the present invention, or their functional derivatives, are well suited for the preparation of pharmaceutical compositions. The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compositions of the invention. Foremost among such animals are humans, although the invention is not intended to be so limited.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The proteins and pharmaceutical compositions can be administered parenterally by bolus injection or by gradual

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perfusion over time.

The dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The dose ranges for the administration of the compositions of the present invention are those large enough to produce the desired effect, whereby, for example, an immune response to the proteins as measured by delayed-type hypersensitivity (DTH) or antibody production, is achieved, and the immune-related disease is significantly prevented, suppressed, or treated. The doses should not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Preferred doses for humans range between about 0.001 - 1 mg/kg body weight.

In addition to proteins of the invention which themselves are pharmacologically active, pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferred compositions include the inclusion of an adjuvant, such as alum, or other adjuvants known in the art. (See, for example, Warren et al., 1986, *Ann. Rev. Immunol.*, 4:369-388; Chedid, L., 1986, *Feder. Proc.*, 45:2531-2560.)

To enhance delivery or bioactivity, the proteins can be incorporated into liposomes using methods and compounds known in the art.

Preparations which can be administered orally in the form of tablets and capsules, preparations which can be administered rectally, such as suppositories, and preparations in the form of solutions for injection or oral introduction, contain about 0.001 to about 99 percent, preferably from about 0.01 to about 95 percent of active compound(s), together with the excipient.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

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Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the proteins in water-soluble form, for example, water-soluble salts. In addition, suspensions of the proteins as appropriate oil injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions containing substances which increase the viscosity of the suspension include, for example, sodium-carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The proteins are formulated using conventional pharmaceutically acceptable parenteral vehicles for administration by injection. These vehicles are nontoxic and therapeutic, and a number of formulations are set forth in Remington's Pharmaceutical Sciences (Martin, E.W., ed.) Mack Publishing Co. Easton, PA (1990). Nonlimiting examples of excipients are water, saline, Ringer's solution, dextrose solution and Hank's balanced salt solution. Formulations according to the invention may also contain minor amounts of additives such as substances that maintain isotonicity, physiological pH, and stability.

The proteins of the invention are preferably formulated in purified form substantially free of aggregates and other protein materials, preferably at concentrations of about 1.0 ng/ml to 100 mg/ml.

Effective doses of the proteins of this invention for use in preventing, suppressing, or treating an immune-related disease are in the range of about 1 ng to 100 mg/kg body weight. A preferred dose range is between about 10 ng and 10 mg/kg body weight. A more preferred dose range between about 100 ng and 1 mg/kg body weight.

Methods to increase the effectiveness of proteins are known in the art. These techniques may be used in conjunction with the proteins of the instant invention.

The following examples are offered by way of illustration and not limitation.

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EXAMPLES

The following general techniques are used throughout the following Examples.

1. GENERAL MATERIALS AND METHODS.

A. Bacteria, plasmids, and general methods

Escherichia coli GM2929, methylase-negative, and *E. coli* LJ24 (*lac* deletion) (Rasmussen et al., 1991, *J. Bacteriology*, 173:6390-6397) were gifts from Dr. Martin Marinus, University of Massachusetts Medical School, Worcester, MA. *E. coli* JM109 (Yanisch-Perron et al., 1985, *Gene*, 33:103-119) were purchased from Promega Corporation, Madison, WI (USA). BL21 (lon^-, $ompT^-$) (Wood, W.B., 1966, *J. Mol. Biol.*, 16:118-133; Studier and Moffatt, 1986, *J. Mol. Biol.*, 189:113-130) were purchased from Novagen, Madison, WI (USA). SG22094 (lon^-, clp^-), SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-) were gifts from Dr. Susan Gottesman, National Institutes of Health, Bethesda, MD (USA). These Gottesman strains have the *lacI* gene deleted. Bacteria were made competent for transformation by the method of Morrison, 1979, *Meth. Enzymology*, 68:326-331) or were purchased (DH5α) from GibcoBRL, Gaithersburg, MD (USA).

pKK233-2 (Amann and Brosius, 1985, *Gene*, 40:183-190) was purchased from Pharmacia, Piscataway, NJ (USA). pBR322 (Bolivar et al., 1977, *Gene*, 2:95-113) was obtained from New England Biolabs, Beverly, MA (USA). pBluescript II KS+ (Alting-Mees et al., 1992, *Meth. Enzymology*, 216:483-495) was obtained from Stratagene, La Jolla, CA (USA). pDC952 containing the *argU* (*dnaY*) gene (Lindsey et al., 1989, *J. Bacteriology*, 171:6197-6205) was a gift from Dr. James R. Walker, University of Texas, Austin, TX. pSE420 (Brosius, 1992, *Meth. Enzymology*, 216:469-483) and pKK480-3 (Brosius, (1992) *supra*) were gifts from Dr. Jürgen Brosius, Mount Sinai School of Medicine, New York, NY. pUCts (Bukrinsky et al., 1988, *Gene*, 70:415-417) was obtained from Michail I. Bukrinsky via Jürgen Brosius.

Plasmid constructions were performed by standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York(1989)). DNA restriction and modification enzymes were purchased from New England Biolabs, unless otherwise stated, and were used according to the manufacturer's specifications. Synthetic oligonucleotides were purchased from Operon Technologies, Inc., Alameda, CA (USA), unless otherwise indicated.

Oligonucleotides were annealed together by mixing equimolar concentrations of the complementary oligonucleotides in 10 mM Tris pH8.0, 5 mM $MgCl_2$ and placing in 100°C for 5 min and then cooling slowly to 25°C. Polymerase chain reaction (PCR) was performed (Saiki et al., 1988, *Science*, 239:487-491) in a Perkin Elmer Cetus DNA Thermal Cycler, using the GeneAmp reagent kit (The Perkin-Elmer Corporation, Norwalk, CT (USA)). DNA sequencing employed the dideoxynucleotide chain termination method (Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA*,

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74:5463-5467) using the Sequenase kit (U.S. Biochemical, Cleveland, OH (USA)). All chemical reagents were of the highest purity available.

B. Growth and induction of cell cultures

For chemically induced cultures, frozen glycerol stocks were used to inoculate 5-25 ml 2XYT medium (16 g Bacto-Tryptone (Difco 0123), 10 g Bacto-Yeast Extract (Difco 0127), 10 g NaCl, pH 7.2-7.4, per liter water) containing 6 μg/ml tetracycline, and allowed to grow overnight at 37°C, 280 rpm. Typically, 1/100 volumes of the overnight culture were used to inoculate each of two 125-ml flasks containing 25 ml 2XYT medium plus 6 μg/ml tetracycline. Cultures were grown at 37°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units, and then induced with 1 mM isopropyl ß-D-thiogalactopyranoside (IPTG), and allowed to grow for a further 2 hr.

For thermal induction, cultures were grown at 30°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units. Thereafter, one culture was maintained at 30°C, and the other was induced by transferring to a water bath at 42°C, and allowed to grow for a further 2 hr. Alternatively, a culture was induced by growing at 30°C until A_{580} was 0.3-0.6 absorbance units, and thereafter was divided into two aliquots; one was maintained at 30°C, and the other was allowed to grow at 42°C.

To assay for results of induction, duplicate samples equivalent to 1 ml of cells at $A_{580} = 1.0$ were removed (e.g., assuming $A_{580} = 3.0$, then 333 μl culture was removed). The samples were centrifuged in an Eppendorf microcentrifuge at 14,000 x g, 1 min., the supernatants discarded, and the cell pellets were stored at -20°C.

C. Stability study

Bacteria were streaked to single colonies three successive times and a single colony from the third plate was used to inoculate 200 ml 2XYT medium containing 6 μg/ml tetracycline. The culture was grown to $A_{580} = 0.5$-1.0 absorbance units, and 60 ml were removed, mixed with 40 ml glycerol and 1 ml aliquots were distributed into 100 vials to be stored at -80°C. The remainder of the culture was divided into two aliquots; one was incubated uninduced, and the other was induced by IPTG addition or temperature shift. Both cultures were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at $A_{580} = 1.0$ were removed prior to and at 2 hr after induction, and assayed for A_{580} absorbance, pH, and protein production by EIA, acrylamide gel electrophoresis, and Western blotting.

Three vials from the frozen bank were tested in parallel for bacterial growth and protein production. Each vial (1 ml) was used to inoculate 200 ml 2XYT medium plus 6 μg/ml tetracycline, and incubated to $A_{580} = 0.5$-1.0 absorbance units. Thereafter each culture was divided into two aliquots, one to be incubated uninduced, the other to be induced chemically or thermally. Samples equivalent to 1 ml culture at $A_{580} = 1.0$ were removed from both cultures prior to and at 2 hr after induction and assayed as described above.

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For the stability study, a 1-ml aliquot was removed from one of the three parallel cultures prior to induction, diluted 1/1000 in 2XYT medium, and 20 µl or 200 µl was inoculated into 200 ml 2XYT medium plus tetracycline, and allowed to grow to A_{580} = 0.5-1.0 absorbance units. Thereafter the culture was divided into two aliquots; one was induced, and both were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed prior to and at 2 hr after induction and assayed as above.

D. Cell fractionation

The cells were pelleted by centrifugation at 7000 x *g*, 7 min, and the supernatant (culture medium) was recentrifuged at 14,000 x *g*, 5 min prior to electrophoresis. The cell pellet was resuspended in 20% (w/v) sucrose, 0.3 M Tris-HCl pH 8.0, 1 mM EDTA using 1/5 of initial culture volume, incubated at 25°C for 15 min, and centrifuged at 6000 x g, 7 min. The supernatant was discarded, and protein located in the periplasm was released by osmotic shock (Nossal and Heppel, 1966, *J. Biol. Chem.*, 241:3055-3062) by resuspending in 1/5 original volume cold water, and incubating at 0°C, 10 min. Following centrifugation at 9000 x *g*, 10 min, the supernatant was saved (periplasmic fraction); the cell pellet was resuspended in 1X TST (25 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, 10 ml/liter Tween 20), sonicated, centrifuged at 12,000 x *g*, 15 min, and the supernatant was saved (soluble cytosolic fraction).

E. Polyacrylamide gel electrophoresis

The frozen *E. coli* cell pellet was resuspended in 70 µl water by vigorous vortexing or by sonication at 0°C (Sonic Dismembrator, Model 301, ARTEK Systems Corporation) three times each for 5 seconds, at power output 0.4. The disrupted cells were mixed with 70 µl 2X loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% ß-mercaptoethanol, 0.002% Bromophenol Blue) (Laemmli, 1970, *Nature*, 227:680-685.) Samples were incubated at 100°C, 5 min., centrifuged at 14,000 x *g*, 30 seconds, and 10-15 µl aliquots were loaded onto pre-cast 16% or 10-20% acrylamide Tricine gels (Novex, San Diego, CA (USA)) and run in reservoir buffer obtained from the same manufacturer. Electrophoresis was at 125 V constant voltage, or 45 mA constant current, per two gels, for about 2 hr, until the bromophenol blue tracking dye was off the gels. Markers were prestained low molecular weight protein standards (# SE130024, Integrated Separation Systems, Natick, MA (USA)) used at 1 µl per lane. Gels were stained overnight in 10% acetic acid, 25% methanol, 0.025% Coomassie Brilliant Blue R.

F. Western blot analysis

Following separation by SDS-PAGE, proteins were immunodetected (Towbin et al., 1979, *Proc. Natl. Acad. Sci. USA*, 76:4350-4354) by electrotransfer to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA (USA)) using a Semi-Dry electroblotter (Integrated Separation Systems). Proteins were transferred at 10 mA per cm^2 membrane for 1-3 hr,

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and the membranes were blocked overnight in blocking buffer (1X PBS, 2% dried milk, 0.1% Tween-20).

The membranes were washed three times, 5 min each in 1X PBS, 0.1% Tween-20, followed by washing three times, 5 min each in 1X PBS, and then they were submerged in an horseradish peroxidase (HRP)-conjugated monoclonal anti-Vβ5.3 antibody (13A2-HRP, 0.25 mg/ml) diluted 1:3000 in 1 X PBS, 0.1% Tween-20. Following gentle agitation for 2 hr, the membranes were washed as described above, and the proteins were visualized with the Enhanced Chemiluminescence assay (Amersham, Arlington Heights, IL (USA)) used according to the manufacturer's instructions. Film exposure was between 5 seconds and 1 minute.

G. Enzyme immunoassay

The frozen cell pellets were resuspended by vigorous vortexing in 20 µl 8 M urea, 50 mM Tris-HCl, pH 8.5, (Marston and Hartley, 1990, *Meth. Enzymology*, 182:264-276) and Vβ5.3 was quantified by enzyme immunoassay as follows:

Nunc-Immuno Maxisorp plates (VWR #62409-002) were coated with 100 µl/well 4C2 IgG at 4 µg/ml in PBS, and stored at 4°C overnight. The coating solution was discarded, and blocking buffer (1% casein hydrolysate in PBS, 0.05% Tween-20) was added at 200 µl/well. The plates were incubated at room temperature for 2 hr, 150 rpm, and then washed three times with wash buffer (PBS, 0.05% Tween-20). Samples (diluted 1/2,000 to 1/32,000) and Vβ5.3 standards in blocking buffer were added at 100 µl/well, and incubated at room temperature for 90 min, 150 rpm. The plates were washed three times with wash buffer, and an HRP-conjugated anti-Vβ5.3 monoclonal antibody (13A2-HRP) diluted 1/500 in blocking buffer plus 5% fetal bovine serum was added at 100 µl/well (0.5 µg/ml). The plates were incubated at room temperature for 90 min, 150 rpm, washed four times, and 100 µl/well O-Phenylenediamine (OPD) substrate was added. After 30 min, the reaction was terminated by adding 50 µl 2N H_2SO_4 per well. The absorbance was measured at 490 nm minus the background absorbance at 650 nm. Blank wells did not contain sample.

2. CONSTRUCTION OF PLASMIDS

EXAMPLE 1

Construction of pK2D

The starting point for the expression of the T cell antigen receptor V region proteins of the invention was the construction of a plasmid that contained the proper regulatory elements for expression of the T cell antigen receptor V region protein. These elements include the promoter, two transcription terminators, an origin of replication for stable replication in bacteria. Additionally because of regulatory concerns associated with the manufacture of a protein for which one possible indication is the *in vivo* use in a therapeutic setting, a gene coding for resistance to tetracycline was chosen to facilitate selection of plasmid bearing cells. Finally, the starting expression vector required a multiple cloning site, strategically engineered to facilitate the cloning of the preferred

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coding regions of the invention into the expression vector. The following example describes the unique starting plasmid of the invention.

The starting point for the construction of preferred expression vector was pKK233-2 (4.6 kb), described *infra*. The *Nco*I restriction site was converted to a *Spe*I site as follows: pKK233-2 was digested with *Nco*I, and the resulting 5' protruding ends were removed by incubation with mung bean nuclease at 25°C for 30 min. The plasmid was extracted twice with phenol-chloroform, precipitated with ethanol in the presence of 2 M ammonium acetate, and resuspended in water. Following dephosphorylation with bacterial alkaline phosphatase (GibcoBRL) the vector was ligated to a synthetic *Spe*I linker (#1085, New England Biolabs), and the ligation mix was transformed into DH5α competent cells to yield vector pKK233-2A.

Next, the tetracycline gene was restored as follows: pKK233-2A (4.6 kb) was digested with *EcoR*I and *Sal*I yielding two fragments, 279 bp and 4321 bp each. The two fragments were separated by agarose gel electrophoresis, and the large fragment was purified from agarose using the Geneclean Kit (BIO 101, La Jolla, CA (USA)). A 651-bp fragment containing the amino-terminal coding region of the tetracycline gene was excised with *EcoR*I and *Sal*I from pBR322, and ligated to the 4321-bp fragment from pKK233-2A. The resulting plasmid pK2B (4.97 kb) conferred tetracycline resistance on *E. coli* DH5α cells, and was further modified to delete a portion of the ß-lactamase gene and convert the *Hind*III site into a *Sac*II site, as follows:

A DNA segment spanning the unique *Spe*I site to the 3' terminus of the transcription terminators was PCR-synthesized using pK2B as template. The 5' "sense" primer contained restriction enzyme recognition sequences for *Spe*I, *Pst*I and *Sac*II in the 5' to 3' direction, underlined:

5' - TATAATGACTAGTCGCTGCAGCCAACCGCGGCTG -3' [SEQ ID NO 1]

The 3' "antisense" primer contained a *Sca*I site, underlined:

5' - GTCCTAGAGTACTGAGCGGATAC - 3' [SEQ ID NO 2]

1 ng of pK2B DNA template was mixed in a reaction volume containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 µg/ml gelatin, 200 µM each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), 1 µM each of the two primers, and 2.5 units AmpliTaq DNA polymerase. The final reaction volume was 100 µl, overlaid with 100 µl mineral oil. The DNA was denatured at 95°C for 2 min, and amplification was achieved with 30 cycles of 1 min at 95°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for primer extension. The amplified DNA was analyzed on 1% agarose gel run in 40 mM Tris-HCl pH 7.8, 5 mM NaAcetate, 1 mM EDTA.

The resulting 485-bp PCR-synthesized fragment was digested with *Spe*I and *Sca*I, and ligated to a 4129-bp fragment that was obtained by digestion of pK2B with *Spe*I and *Sca*I resulting in a plasmid called pK2C.

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Plasmid pK2C (4.6 kb) was further modified to contain a multiple cloning site. First, pK2C was digested with *Spe*I and *Sac*II, and ligated to a double-stranded synthetic oligonucleotide containing 15 restriction sites. This multiple cloning site (MCS) was formed by annealing equimolar concentrations of two complementary synthetic oligonucleotides, 91 and 97 bp long, respectively, having the following sequences:

5' - GGCTCGAGCCTAGGCTGCAGCCCGGGGCGCGCGCGGCCGCAGGCC
TTTAATTAAGAGCTCCGGACCGCACAATGTGGGCGCGCCCTTAAGA - 3'
[SEQ ID NO 3]

5' - CTAGTCTTAAGGGCGCGCCCACATTGTGCGGTCCGGAGCTCTTAA
TTAAAGGCCTGCGGCCGCGCGCGCCCCGGGCTGCAGCCTAGGCTCGAG
CCGC - 3' [SEQ ID NO 4]

The MCS codes for the following restriction enzymes sites: *Spe*I, *Afl*II, *Asc*I, *Dra*III, *Rsr*II, *Sac*I, *Pac*I, *Stu*I, *Not*I, *BssH*II, *Xma*I, *Pst*I, *Avr*II, *Xho*I, and *Sac*II. The ligation mix was transformed into methylase-negative *E. coli* GM2929. The resulting plasmid pK2D (4.67 kb) was partially sequenced to ensure the integrity of the *in vitro* synthesized regions.

Results

The starting plasmid was pKK233-2 which contains the following elements: The *trc* promoter (Brosius et al., (1985) *supra*) which is similar to the *tac* promoter derived by fusing the -10 region of the *lac*UV5 promoter with the -35 region of the tryptophan promoter (de Boer et al., (1983) *supra*; Amann et al., (1983) *supra*). The plasmid also contains a ribosome binding site followed by a cloning site of three unique restriction enzymes (*Nco*I, *Pst*I and *Hind*III), followed by the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118). In addition, pKK233-2 contains the ß-lactamase gene for ampicillin resistance, the pBR322 origin of replication, and a small non-functional carboxyl terminal fragment from the tetracycline resistance gene.

pKK233-2 was modified in order to destroy the *Nco*I site, restore the tetracycline resistance gene, delete a segment of the ß-lactamase coding region (thus inactivating the gene), and insert a multiple cloning site consisting of 15 restriction sites to produce pK2D.

EXAMPLE 2

Plasmid pK2D was further modified to incorporate either the pelB signal sequence from *Erwinia carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383) or the heat stable enterotoxin II (STII) signal sequence of *Escherichia coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732.)

Construction of pK-pelB.

Plasmid pK2D (4.67 kb) was engineered to include a ribosome binding site (Shine and Dalgarno, 1974, *Proc. Natl. Acad. Sci. USA*, 71:1342-1346; Shine and Dalgarno, 1975, *Nature*,

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254:34-38; Steitz and Jakes, 1975, *Proc. Natl. Acad. Sci. USA*, 72:4734-4738) and the pelB signal sequence from *E. carotovora* (Lei et al., (1987) *supra*) to create plasmid pK-pelB (4.74 kb), as follows:

pK2D was digested with *Spe*I and *Sac*I located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 103 bp and 95 bp long, respectively, having the sequence shown below. A silent mutation (lower case letters) was introduced into the pelB sequence, in order to create an *Nco*I restriction site, underlined, facilitating the subsequent subcloning of the Vβ5.3 gene sequence.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAATACC
TATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCA
GCcATGGCCGAGCT - 3' [SEQ ID NO 5]

5' - CGGCCATgGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCT
GCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGAAATAGA
ATTTA - 3' [SEQ ID NO 6]

Construction of pK-STII.

Plasmid pK2D was engineered to include a ribosome binding site and the heat-stable enterotoxin (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., (1991) *supra*), as follows:

pK2D was digested with *Spe*I and *Dra*III located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 105 bp and 98 bp long, respectively, having the sequence shown below. Two silent mutations (lower case letters) were introduced into the STII sequence, in order to create *Mlu*I and *Dra*III restriction sites , underlined, facilitating the subsequent subcloning of Vβ5.3.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAAAAGAA
TATAGCATTCCTACTAGCTTCAATGTTCGTCTTCTCTATTGCAACTA
ACGCgTACGCaCATT - 3' [SEQ ID NO 7]

5' - GtGCGTAcGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGCT
AGTAGGAATGCTATATTCTTTTTCATTATGACTGTCTCCTTGAAATA
GAATTTA - 3' [SEQ ID NO 8]

EXAMPLE 3

The plasmids containing the signal sequences from Example 2 *supra* were further modified to include two regulatory elements.

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Construction of pKT-pelB.

Plasmid pK-pelB was further modified to include two regulatory elements upstream of the pelB leader, to create pKT-pelB (4.8 kb). These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron, ATG TAT CGA TTA AAT AAG GAG GAA TAA (SEQ ID NO.28), coding for 8 amino acid residues. Insertion of the two regulatory elements was achieved by digestion of pK2D with *Spe*I and *Sac*I, followed by ligation of the vector to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the pelB leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 71-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp, respectively) and Pair B (oligos J5B + J6B, 71 bp and 76 bp, respectively) having the following sequence:

J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA
AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA
ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5B

5' - ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTC
GCTGCCCAACCAGCCATGGCCGAGCT - 3' [SEQ ID NO 11]

J6B

5' - CGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCTG
CCGTAGGCAATAGGTATTTCATTATTTATTC - 3' [SEQ ID NO 12]

Construction of pKT-STII.

Plasmid pK-STII was further modified to include the same two regulatory elements as in pKT-pelB, thus creating pKT-STII (4.8 kb). pK2D was digested with *Spe*I and *Dra*III, and the vector was ligated to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the STII leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 73-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp, respectively) and Pair B (oligos J5C + J6C, 73 bp and 79 bp, respectively) having the following sequence:

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J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA

AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA

ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5C

5' - ATGAAAAAGAATATAGCATTCCTACTAGCTTCAATGTTCGTCTT

CTCTATTGCAACTAACGCGTACGCACATT - 3' [SEQ ID NO 13]

J6C

5' - GTGCGTACGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGC

TAGTAGGAATGCTATATTCTTTTTCATTATTTATTC - 3' [SEQ ID NO 14]

EXAMPLE 4

Construction of pK-pelB-Vβ5.3.

The next step in the expression of the T cell antigen receptor Vβ5.3 protein was the incorporation of the Vβ5.3 sequence into the plasmids containing the signal sequences as described in Example 2 *supra*.

The complete nucleotide sequence of Vβ5.3 was generated as follows: the DNA sequence of Vβ5.3 clone 12A1 was originally published by Leiden et al. (1986, *Proc. Natl. Acad. Sci. USA*, 83:4456-4460) and later partially corrected (Leiden et al., 1986, *Molec. Cell Biol.*, 6:3207-3214). This clone 12A1 was missing nucleotides coding for the N-terminal four amino acid residues; and in addition, the deduced N-terminal amino acid residues of clone 12A1 were incorrect. The N-terminal amino acid sequence of Vβ5.3 (HPB-ALL) was published by Jones et al., 1985, *Science*, 227:311-314) without specifying the N-terminal first amino acid residue, which was later determined to be Glycine (Jeff Leiden, unpublished). Using the above sequence information, the complete Vβ5.3 was reconstructed by recombinant DNA techniques. The resulting plasmid (pBB-Vβ5.3) was the source of the Vβ5.3 sequence for the following examples. The full length nucleotide sequence coding for Vβ5.3 and the predicted amino acid sequence have been published more recently by Plaza et al. (1991, *J. Immunol.*, 147:4360-4365). (See, also, Figure 1; SEQ ID NOS. 25, 26 and 27 herein.)

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TAATTAGCCATGGCCGGCGTAACCCAATCTCCG - 3' [SEQ ID NO 15]

The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 16]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *Eco*RV site of pBluescript II KS+ to yield plasmid pBL-pelB-

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Vβ5.3. The Vβ5.3 insert was excised by digestion with *Nco*I and *Pst*I, purified from agarose, and ligated into the *Nco*I and *Pst*I sites of pK-pelB, yielding plasmid pK-pelB- Vβ5.3 (5 kb).

Construction of pK-STII-Vß5.3.

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Mlu*I restriction enzyme site, underlined:

5' - GAAATTAACGCGTACGCAGGCGTAACCCAATCTC - 3' [SEQ ID NO 17]

The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 18]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *EcoR*V site of pBluescript II KS+ to yield plasmid pBL-STII-Vβ5.3. The Vβ5.3 insert was excised by digestion with *Mlu*I and *Pst*I, purified from agarose, and ligated into the *Mlu*I and *Pst*I sites of pK-STII, yielding plasmid pK-STII- Vβ5.3 (5 kb).

Results

Vβ5.3 fused to the pelB or the STII leaders was expressed in *E. coli* JM109 (TABLE 1). Induction was for 2 hr. and Vβ5.3 yields were measured by EIA.

TABLE 1

EXPRESSION AND COMPARTMENTATION OF Vβ5.3

	pK-pelB-Vß5.3		pK-STII-Vß5.3	
Compartment	0 mM IPTG	1 mM IPTG	0 mM IPTG	1 mM IPTG
Supernatant	0 µg/ml	1.3 µg/ml	0.5 µg/ml	2.2 µg/ml
Periplasm	0.9	4.6	N.D.	5.5
Cytosol	9.9	Off scale [1:40]	2.0	Off scale [1:40]

Both leaders were functional, although most of the expressed protein was localized intracellularly.

EXAMPLE 5

This Example details the plasmids containing the leader sequence and the two regulatory elements (g10 and mini-cistron) as well as the Vβ5.3 sequence.

Construction of pKT-pelB-Vß5.3

Plasmid pKT-pelB-Vβ5.3 was constructed by excision of the Vβ5.3 insert from pK-pelB-Vβ5.3 using *Nco*I and *Pst*I, and ligation into the *Nco*I and *Pst*I sites of pKT-pelB.

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Construction of pKT-STII-Vß5.3

Plasmid pKT-STII-Vβ5.3 was similarly constructed by excision of the Vβ5.3 insert from pK-STII-Vβ5.3 using *Mlu*I and *Pst*I, and ligation into the *Mlu*I and *Pst*I sites of pKT-STII.

All subsequent plasmid constructs utilized the STII signal sequence. Therefore, where the signal sequence is not specified, it is understood that the STII sequence was utilized.

Results

pKT-Vß5.3

Plasmid pK-STII-Vβ5.3 (Example 4) was modified to include two regulatory elements upstream of the STII leader, to create pKT-Vβ5.3. These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron coding for 8 amino acid residues. Both elements have been demonstrated to enhance translational efficiency of several genes (Schoner et al., 1984, *Proc. Natl. Acad. Sci. USA*, 81:5403-5407, Schoner et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:8506-8510; Olins et al., 1988, *Gene*, 73:227-235; Olins and Rangwala, 1989, *J. Biol. Chem.*, 264:16973-16976).

pKT-Vβ5.3 provided up to 8-fold higher protein yields than pK-STII-Vβ5.3 (Example 4) in *E. coli* JM109 under conditions of IPTG induction. The EIA values reported here are approximations, due to the lack of a pure Vβ5.3 standard at this time. pKT-Vβ5.3 was shown to be stable in *E. coli* JM109.

EXAMPLE 6

Construction of pKTB-Vß5.3.

A further modification to pKT-Vβ5.3 (Example 5) for secreted expression involved elimination of the IPTG requirement for induction. The existence of a *lacI*ts gene coding for temperature-sensitive *lac* repressor (Bukrinsky et al., 1988, *Gene*, 70:415-417) makes it possible to control gene expression from the *trc* promoter by elevating the temperature from 30°C to 42°C. This would obviate the need to use IPTG or bacterial strains harboring the *lacI* or *lacI*^{q} genes.

The *lacI*ts gene was excised from pUCts by *EcoR*I digestion. The 1.7 kb fragment containing the entire *lacI*ts-coding region and its associated regulatory elements, flanked by vector sequence, was blunt-ended with Klenow, and ligated into the dephosphorylated ScaI site, located downstream of the rrnB operon in pKT-Vβ5.3, yielding plasmid pKTB-Vβ5.3 (6.8 kb). The orientation of the *lacI*ts gene was determined by restriction mapping.

Results

The *lacI*ts gene was inserted in either orientation downstream of the *rrnB* transcription terminators in pKT-Vβ5.3 to create plasmid pKTB-Vβ5.3 (Fig. 2). This was expressed in *E. coli* LJ24 (*lacI* deletion) and the protein was analyzed by Coomassie Blue staining on acrylamide gels (Laemmli, (1970) *supra*) and by Western blot (Towbin et al., (1979) *supra*). One

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orientation of the *lacIts* gene (transcription direction toward the Amp^r region) clearly resulted in higher Vβ5.3 yield than the reverse orientation.

The putative Vβ5.3 band obtained from extracts of total cellular pellets was electrotransferred to Immobilon-P membranes, and visualized by Coomassie Blue staining. The band was excised and subjected to NH_2-terminal amino acid sequencing (Matsudaira, 1987, *J. Biol. Chem.*, 262:10035-10038) to yield a total of 10 amino acid residues. These matched precisely the sequence of the N-terminal 10 amino acid residues of the processed Vβ5.3, i.e. no leader sequence was detected.

This plasmid was also expressed by thermal induction in three different bacterial strains, each of which contains increasing levels of endogenous *lac* repressor, i.e., LJ24<DH5α<JM109. Supernatants from induced cultures were assayed for Vβ5.3 expression by EIA (Table2)

TABLE 2

pKTB-Vβ5.3 EXPRESSION IN DIFFERENT BACTERIAL STRAINS

Cells were induced for 2 hr., and Vβ5.3 yields (μg/ml) were measured in culture supernatants.

Plasmid	Regulation	*E. coli* Host	Induction	Uninduced	Induced
pKTB-Vβ5.3	*lacIts*	JM109 (lacI^q)	42°C	N.D.	0.5
pKTB-Vβ5.3	*lacIts*	DH5α (lacI)	42°C	N.D.	5.0
pKTB-Vβ5.3	*lacIts*	LJ24 (Δ*lacI*)	42°C	N.D.	5.2
pKT-Vβ5.3	*lacI*^{q}	JM109	2mM IPTG	0	0.9
pKT	*lacI*^{q}	JM109	42°C	N.D.	0

The data agree with Western blot observations and predicted results, i.e., the highest expression level was achieved in LJ24 (Δ*lacI*) and the lowest in JM109 (*lacI*^{q}). The comparatively low amounts of Vβ5.3 measured in the culture supernatants follow from the fact that *E. coli* does not ordinarily secrete proteins into the medium. As shown in Table 1, the bulk of the expressed Vβ5.3 is retained in the periplasm.

DNA sequencing of the *lacIts* gene.

The entire coding sequence of the temperature-inducible *lacIts* gene plus the upstream 80 nucleotides including the promoter were sequenced and compared to the published sequence of the IPTG-inducible *lacI* gene (Farabaugh, 1978, *Nature*, 274:765-769). The promoter region was identical to that of the *lacI*, and not *lacI*^{q}, gene (Calos M. P., 1978, *Nature*, 274:762-765). The *lacIts* gene sequence showed an open reading frame of 1080 nucleotides encoding 360 amino acids, and differed from

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the *lacI* gene at nucleotide #559 (with reference to first nucleotide of the start codon), which changed from G to A, causing amino acid residue #187 to change from Glycine (GGC) to Serine (AGC). This change in a single amino acid residue imparts thermal sensitivity to the *lac* repressor protein.

In addition, the published sequence of the *lacI* gene (Farabaugh, (1978) *supra*) as well as the sequence appearing in Genebank (locus ECOLAC) contains an error at position #857: nucleotide #857 should be T instead of C, which would make residue #286 code for Leucine (TTA) instead of Serine (TCA). The C-T transition introduces a *Hpa*I site at the 3' end of the *lacI* gene that was shown previously by restriction mapping (Brosius, 1992, *Meth. Enzymol.*, 216:469-483). It should be noted that in Farabaugh, (1978) *supra*, the same codon (TCA) is erroneously reported as encoding Leucine instead of Serine.

EXAMPLE 7

Construction of pKTB.

Plasmid pKTB-Vβ5.3 (6.7 kb) was digested with *Sal*I and *Pst*I, and the 5.42 kb fragment was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pKT-STII (4.8 kb) was digested with *Sal*I and *Pst*I, and the 1.15 kb fragment was similarly purified, and ligated to the 5.42 kb fragment, yielding plasmid pKTB (6.56 kb).

EXAMPLE 8

Construction of pKB-Vß5.3.

This plasmid is identical to pKTB-Vβ5.3 minus the two regulatory elements, i.e., the g10 translational enhancer and the mini-cistron. Plasmid pKTB (6.56 kb) was digested with *Sal*I and *Pst*I, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-Vβ5.3 (4.9 kb) was digested with *Sal*I and *Pst*I, and the 1.3-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB-Vβ5.3 (6.7 kb).

Results

pKB-Vß5.3

As discussed above, the addition of two regulatory elements, i.e., the g10 gene segment and the mini-cistron, appeared to enhance Vβ5.3 yield under conditions of IPTG induction. We wished to remove these two elements from the pKTB-Vβ5.3 vector, and reexamine Vβ5.3 yield under conditions of thermal induction. A comparison of pKTB-Vβ5.3 and pKB-Vβ5.3 by acrylamide electrophoresis and Western blotting showed that Vβ5.3 yield was unaffected by the removal of these two elements. EIA measurements of whole cell urea extracts showed that pKB-Vβ5.3 produced more protein than pKTB-Vβ5.3 when expressed in *E. coli* LJ24.

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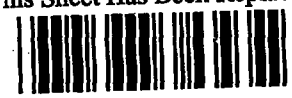


TABLE 3

EXPRESSION OF Vβ5.3 IN DIFFERENT BACTERIAL STRAINS

Plasmid	LJ24	SG22094	SG21163	SG21173
pKTB-Vß5.3 + leader + g10/MC	90	80	20	30
pKB-Vß5.3 + leader - g10/MC	110	50	40	20
pKTBi-Vß5.3 - leader + g10/MC	6	100	100	100
pKBi-Vß5.3 - leader -g10/MC	10	170	130	390

Induction period: 2 hr at 42°C.
Culture volume: 25 ml
Leader: STII
g10: T7 gene 10
MC: Mini-cistron
Samples: whole cell urea extracts
Vβ5.3 yields (mg/liter), based on units/ml approximations, as measured by EIA. 1 mg Vβ5.3 is approximately equivalent to 1 x 10^7 units.

Plasmid pKB-Vβ5.3 was demonstrated to be stable for fermentation.

EXAMPLE 9

Construction of pKB

Plasmid pKTB (6.56 kb) was digested with *Sal*I and *Pst*I, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-STII (4.75 kb) was digested with *Sal*I and *Pst*I, and the 1.1-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB (6.5 kb).

EXAMPLE 10

Construction of pKTBi.

This plasmid is identical to pKTB minus the STII signal sequence. pKTB (6.56 kb) was digested with *Spe*I and *Xma*I, and ligated to a double-stranded synthetic oligonucleotide coding for the g10 translational enhancer, a ribosome binding site, and a mini-cistron containing a second ribosome binding site. Restriction sites for *Nco*I, *Dra*III, *Rsr*II and *Xma*I were designed in the 3' end. The two complementary oligonucleotides had the following sequences:

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5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTA
AAGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAACC
ATGGCACATTGTGCGGTCCGC - 3' [SEQ ID NO 19]

5' - CCGGGCGGACCGCACAATGTGCCATGGTTTATTCCTCCTTATTTA
ATCGATACATTAATATATACCTCTTTAATTTTTAATAATAAAGTTA
ATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 20]

EXAMPLE 11

Construction of pKBi

This plasmid is identical to pKB minus the STII signal sequence. pKTB (6.56 kb) was digested with *Spe*I and *Dra*III, and ligated to a double-stranded synthetic oligonucleotide containing a ribosome binding site, and *Nco*I and *Dra*III sites designed in the 3' end. The two complementary oligonucleotides had the following sequences:

5' - CTAGTAAATTATATTTAAAGGAGGAATAAACCATGGCACATT - 3' [SEQ ID NO 21]

5' - GTGCCATGGTTTATTCCTCCTTTAAATATAATTTA - 3' [SEQ ID NO 22]

EXAMPLE 12

Construction of pKTBi-Vß5.3

This plasmid is identical to pKTB-Vß5.3 minus the STII signal sequence. pKTBi (6.4 kb) was digested with *Xma*I and *Nco*I and ligated to PCR-synthesized Vβ5.3 using pKTB-Vβ5.3 as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TATAGTCCATGGGCGTAACC - 3' [SEQ ID NO 23]

The 3' "antisense" primer contained an *Xma*I site, underlined:

5' - AACTTCCCGGGTTATCATTAGCTGC - 3' [SEQ ID NO 24]

Results

pKTBi-Vß5.3

In parallel with the above work, the STII leader was removed from the expression plasmid. Since most of the expressed Vβ5.3 is retained intracellularly (also see below), it was expected that scale-up of this construct might yield a heterogeneous population of Vβ5.3 molecules, i.e., plus and minus leader, which would complicate subsequent purification of the processed Vβ5.3. Therefore, the STII leader was removed to create pKTBi-Vβ5.3 for

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intracellular expression. The expressed Vβ5.3 is expected to contain an N-terminal methionine, although this amino acid might be removed by endogenous methionine aminopeptidases (Ben-Bassat et al., 1987, *J. Bacteriology*, 169:751-757; Baneyx and Georgiou, "Expression of Proteolytically Sensitive Polypeptides in *Escherichia coli*", in Stability of Protein Pharmaceuticals, Part A: Chemical and Physical Pathways of Protein Degradation (Tim J. Ahern and Mark C. Manning, eds.), Plenum Press, New York, pp. 69-108 (1992)), as observed for pKBi-Vβ5.3 (below).

pKTBi-Vβ5.3 was completed, but it failed to express significant levels of protein in *E. coli* LJ24 (see Table 3, *supra*). The entire Vβ5.3 insert was sequenced and determined to be unambiguously correct. Plasmid DNA isolated post-induction and analyzed by restriction digestion appeared to be unaltered. One possible explanation for the apparent failure of the LJ24 strain containing pKTBi-Vβ5.3 to express significant levels of Vβ5.3 protein is that the absence of a leader peptide destabilizes intracellular Vβ5.3 protein, and leads to its rapid proteolysis.

Therefore, pKTBi-Vβ5.3 was transformed into the protease-deficient strains SG22094 (lon^-, clp^-), and BL21 (lon^-, $ompT^-$) which gave decreasing yields in the order of SG22904>BL21. The effect of different induction temperatures on yield was also examined, and for both strains 42°C was optimal. In addition, there was no significant increase in production between 2 and 5 hr.

pKTBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-). The results are summarized in Table 3 (*supra*).

It should be noted that strains LJ24, SG22094, SG21163 and SG21174 do not produce endogenous *lac* repressor because the *lacI* gene is deleted in all of these strains. Thus, the wild type *lac* repressor does not interfere with the thermal induction of the *trc* promoter. The foregoing examples show that Vβ5.3 constucts without a signal peptide can be readily expressed in protease deficient hosts, resulting in production of significant amounts of Vβ5.3 protein.

EXAMPLE 13

Construction of pKBi-Vβ5.3

This plasmid is identical to pKTBi-Vβ5.3 minus the g10 element and the mini-cistron (SEQ ID NO. 28). Plasmid pKBi (6.43 kb) was digested with *Nco*I and *Sal*I, and a 970 bp fragment was purified from agarose. pKTBi-Vβ5.3 (6.75 kb) was digested with *Nco*I and *Sal*I and the 5.68 kb fragment was similarly purified, and ligated to the 970 bp fragment, yielding plasmid pKBi-Vβ5.3 (6.64 kb).

Results

pKBi-Vβ5.3

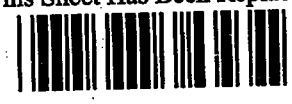
This leaderless Vβ5.3 construct minus the two regulatory elements was successfully expressed in protease-deficient strains; our observations are similar to those for pKTBi-Vβ5.3.

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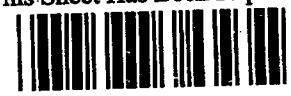
pKBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon⁻, htpr⁻) and SG21173 (lon⁻, htpr⁻, clp⁻). The results are summarized in Table 3. pKBi-Vβ5.3 consistently produced more protein than pKTBi-Vβ5.3 in all strains tested (Table 3). N-terminal sequencing of the expressed protein showed that the first 17 amino acid residues matched the expected Vβ5.3 sequence, and the terminal methionine residue was absent. The plasmid was also demonstrated to be stable.

The present invention is not meant to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modification are intended to fall within the scope of the appended claims. Various publications are incorporated by reference in their entirety.

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SEQUENCE LISTING

(1)GENERAL INFORMATION:

(i)APPLICANT:T Cell Sciences, Inc

(ii)TITLE OF INVENTION:T Cell Antigen Receptor V Region Proteins and Methods of Preparation Thereof

(iii)NUMBER OF SEQUENCES:29

(iv)CORRESPONDENCE ADDRESS:
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(B)STREET:115 Fourth Avenue
(C)CITY:Needham
(D)STATE:Massachusetts
(E)COUNTRY:United States of America
(F)ZIP:02194-2725

(v)COMPUTER READABLE FORM:
(A)MEDIUM TYPE:Diskette, 3.50 inch, 1.44Mb storage
(B)COMPUTER:IBM PC Compatible
(C)OPERATING SYSTEM:MS-DOS
(D)SOFTWARE:WordPerfect 6.0

(vi)CURRENT APPLICATION DATA:
(A)APPLICATION NUMBER:
(B)FILING DATE:
(C)CLASSIFICATION:

(vii)PRIOR APPLICATION DATA:
(A)APPLICATION NUMBER:08/181,492
(B)FILING DATE:13-January-1994

(viii)ATTORNEY/AGENT INFORMATION:
(A)NAME:YANKWICH, Leon R
(B)REGISTRATION NUMBER:30,237
(C)REFERENCE/DOCKET NUMBER:TCS-203-PCT(94,664-A)

(ix)TELECOMMUNICATION INFORMATION:
(A)TELEPHONE:617-345-9100
(B)TELEFAX:617-345-9111

(2)INFORMATION FOR SEQ ID NO: 1:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:34 base pairs
(B)TYPE:nucleic acid
(C)STRANDEDNESS:single
(D)TOPOLOGY:linear

(ii)MOLECULE TYPE:other nucleic acid
(A)DESCRIPTION:synthetic oligonucleotide

(iii)HYPOTHETICAL:

(iv)ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TATAATGACT AGTCGCTGCA GCCAACCGCG GCTG 34

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:23 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTCCTAGAGT ACTGAGCGGA TAC 23

(2) INFORMATION FOR SEQ ID NO: 3:

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(A) LENGTH:91 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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TTAAGAGCTC CGGACCGCAC AATGTGGGCG CGCCCTTAAG A 91

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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GGCCTGCGGC CGCGCGCGCC CCGGGCTGCA GCCTAGGCTC GAGCCGC 97

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAATA CCTATTGCCT 50

ACGGCAGCCG CTGGATTGTT ATTACTCGCT GCCCAACCAG CCATGGCCGA 100

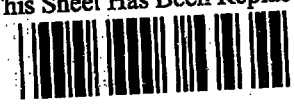
GCT 103

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

GGCAATAGGT ATTTCATTAT GACTGTCTCC TTGAAATAGA ATTTA 95

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:105 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAAAA GAATATAGCA 50

TTCCTACTAG CTTCAATGTT CGTCTTCTCT ATTGCAACTA ACGCGTACGC 100

ACATT 105

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:98 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATGACTGTC TCCTTGAAAT AGAATTTA 98

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:89 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAATA 89

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:76 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

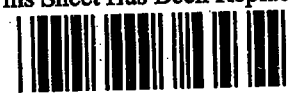
(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTCCTTATTT AATCGATACA TTAATATATA CCTCTTTAAT TTTTAATAAT 50

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AAAGTTAATC GATGCCCAAT TCCGGA 76

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:71 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGAAATACC TATTGCCTAC GGCAGCCGCT GGATTGTTAT TACTCGCTGC 50

CCAACCAGCC ATGGCCGAGC T 71

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:76 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

GGCAATAGGT ATTTCATTAT TTATTC 76

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:73 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single

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(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATGAAAAAGA ATATAGCATT CCTACTAGCT TCAATGTTCG TCTTCTCTAT 50

TGCAACTAAC GCGTACGCAC ATT 73

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:79 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATTTATTC 79

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:33 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAATTAGCCA TGGCCGGCGT AACCCAATCT CCG 33

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:34 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:
(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GAAATTAACG CGTACGCAGG CGTAACCCAA TCTC 34

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid

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(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAACCA TGGCACATTG 100

TGCGGTCCGC 110

(2) INFORMATION FOR SEQ ID NO: 20:

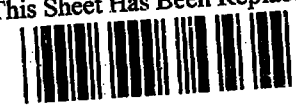
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CCGGGCGGAC CGCACAATGT GCCATGGTTT ATTCCTCCTT ATTTAATCGA 50

TACATTAATA TATACCTCTT TAATTTTTAA TAATAAAGTT AATCGATGCC 100

CAATTCCGGA 110

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTAGTAAATT ATATTTAAAG GAGGAATAAA CCATGGCACA TT 42

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

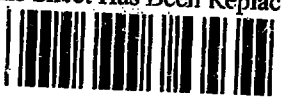
(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTGCCATGGT TTATTCCTCC TTTAAATATA ATTTA 35

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(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:20 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TATAGTCCAT GGGCGTAACC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:25 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AACTTCCCGG GTTATCATTA GCTGC 25

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

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(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA 39

GGA CAG CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG 78

CAC AAG AGT GTG TCC TGG TAC CAA CAG GTC CTG GGT CAG 117

GGG CCC CAG TTT ATC TTT CAG TAT TAT GAG AAA GAA GAG 156

AGA GGA AGA GGA AAC TTC CCT GAT CGA TTC TCA GCT CGC 195

CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT GTG AAC GCC 234

TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC AGC 273

AGC 276

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CCG CAT TGG GTT AGA GGC TGA GTG GAC TAG TTT TGC TCT 39

CCT GTC GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC 78

GTG TTC TCA CAC AGG ACC ATG GTT GTC CAG GAC CCA GTC 117

CCC GGG GTC AAA TAG AAA GTC ATA ATA CTC TTT CTT CTC 156

TCT CCT TCT CCT TTG AAG GGA CTA GCT AAG AGT CGA GCG 195

GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA CAC TTG CGG 234

AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG TCG 273

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TCG 276

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:92 amino acids
(B) TYPE:amino acid
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:protein

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln
 1               5                  10                  15
His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val
            20                  25                  30
Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe
            35                  40                  45
Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp
            50                  55                  60
Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn
            65                  70                  75
Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala
            80                  85                  90
Ser Ser
    92
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:27 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

ATG TAT CGA TTA AAT AAG GAG GAA TAA 27

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(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:8 amino acids
(B) TYPE:amino acid
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Met Tyr Arg Leu Asn Lys Glu Glu
1 5 8

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We claim:

1. A method for producing a recombinant full length T cell antigen receptor V region protein which comprises;

a) transforming a competent bacterial host cell with a recombinant expression vector comprising a region coding for a full length T cell antigen receptor V region protein, and an inducible bacterial promoter;

b) inducing the inducible bacterial promoter in the transformed bacterial host cell of step a, and;

c) recovering the recombinant full length T cell antigen receptor V region protein.

2. The method of claim 1 wherein the inducible bacterial promoter is selected from the group consisting of chemically regulated promoters and thermally regulated promoters.

3. The method of claim 2 wherein the expression vector further comprises a regulatory gene sequence selected from the group consisting of the *lacI* gene or the *lacI*^{q} gene.

4. The method of claim 2 wherein the expression vector includes the *lacI*ts gene.

5. The method of claim 4 wherein the *lacI*ts gene is in the same orientation as the coding region for T cell antigen receptor V region protein.

6. The method of claim 1 wherein the bacterial promotor is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

7. The method of claim 6 wherein the recombinant expression vector further comprises a leader sequence.

8. The method of claim 7 wherein the leader sequence is selected from the group consisting of heat stable enterotoxin STII leader from *Escherichia coli* and a pelB leader from *Erwinia carotovora*.

9. The method of claim 8 wherein the recombinant expression vector further comprises a leader sequence derived from the STII leader sequence of *E. coli* and the competent bacterial host cell is a cell which does not harbor the *lacI* or *lacI*^{q} gene.



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10. The method of claim 9 wherein the bacterial host strain is a protease deficient strain.

11. The method according to claim 10, wherein the protease deficient strain is selected from the group consisting of SG22094, SG21163, and SG21173.

12. The method according to claim 1, wherein the expression vector further comprises the g10 and the mini-cistron sequence (SEQ ID NO. 28) regulatory elements.

13. The method of claim 1 wherein the recombinant expression vector further comprises;

a) a *trc* promotor as the inducible bacterial promoter;

b) a ribosomal binding site;

c) a transcription terminator;

d) a *lacI*ts regulatory gene sequence;

e) a pBR322 origin of replication, and;

f) a tetracycline resistance gene.

14. The method of claim 13 wherein the recombinant expression vector is selected from the group consisting of pKTBi-Vß5.3, and pKBi-Vß5.3.

15. The method of claim 13 wherein the recombinant expression vector further comprises a leader sequence.

16. The method of claim 15 wherein the recombinant expression vector is selected from the group consisting of pK-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, and pKB-Vß5.3.

17. An expression vector comprising a inducible bacterial promoter under the control of a *lacI*ts gene, wherein said *lacI*ts gene controls the expression of a region encoding a full length T cell antigen receptor V region protein.

18. The expression vector of claim 17 wherein the inducible bacterial promoter is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

19. The expression vector of claim 17 wherein the inducible bacterial promoter is the *trc* promoter.

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20. The expression vector of claim 19 which further comprises a leader sequence.

21. An expression vector which comprises in 5' to 3' direction;

a) a *trc* promotor;

b) a ribosomal binding site;

c) a region encoding a full length T cell antigen receptor V region protein;

d) a transcription terminator;

e) a *lacI*ts regulatory gene sequence;

f) a pBR322 origin of replication, and;

g) a tetracycline resistance gene.

22. A bacterial cell transformed with the expression vector according to claim 17.

23. The expression vector of claim 17 wherein said vector is selected from the group consisting of pK-STII-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, pKTBi-Vß5.3, and pKBi-Vß5.3.

24. A full length T cell antigen receptor V region protein produced by the method of claim 13.

25. A pharmaceutical composition which comprises a full length T cell antigen receptor V region protein.

26. The pharmaceutical composition of claim 25 wherein the T cell antigen receptor V region protein is the Vß5.3 protein and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.

27. A method for treating an immune related disorder comprising administering to a subject in need of such treatment a pharmaceutical composition comprising a full length T cell antigen receptor V region protein.

28. The method according to claim 27 wherein the immune related disorder is multiple sclerosis.

29. The method of claim 28 wherein the full length T cell antigen receptor V region protein is the Vß5.3 and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.

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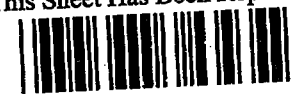
30. A substantially pure T cell antigen receptor Vβ5.3 protein having the formula: amino acids 1-92 of amino acid Sequence ID No. 27.

31. The use of a substantially pure T cell antigen receptor V region protein in the manufacture of a medicament for treatment of an immune-related disease.

32. A transformed bacterial cell which produces at least 300 milligrams of full length recombinant T cell antigen receptor Vβ protein per liter of cell culture.

33. A method of diagnosing an immune disorder comprising using a recombinant full length T cell antigen receptor V region protein to detect in a biological sample from a patient the presence of a particular subpopulation of T cells associated with an immune disorder or the presence of autoantibodies recognizing the particular V region protein.

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ABSTRACT

The present invention relates to the production of full length T cell antigen receptor V region proteins. The invention provides for methods of expressing the proteins as well as expression vectors for enhanced production of full length T cell antigen receptor V region proteins. The invention further provides for the Vβ5.3 region protein. The proteins of the invention have uses in the diagnosis and therapy of immune-related disorders.

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Figure 1

```
  1  GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA GGA CAG
     CCG CAT TGG GTT AGA GGC TGA CTG GAC TAG TTT TGC TCT CCT GTC
  1  Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln

 46  CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG CAC AAG AGT GTG
     GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC GTG TTC TCA CAC
 16  His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val

 91  TCC TGG TAC CAA CAG GTC CTG GGT CAG GGG CCC CAG TTT ATC TTT
     AGG ACC ATG GTT GTC CAG GAC CCA GTC CCC GGG GTC AAA TAG AAA
 31  Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe

136  CAG TAT TAT GAG AAA GAA GAG AGA GGA AGA GGA AAC TTC CCT GAT
     GTC ATA ATA CTC TTT CTT CTC TCT CCT TCT CCT TTG AAG GGA CTA
 46  Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp

181  CGA TTC TCA GCT CGC CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT
     GCT AAG AGT CGA GCG GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA
 61  Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn

226  GTG AAC GCC TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC
     CAC TTG CGG AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG
 76  Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala

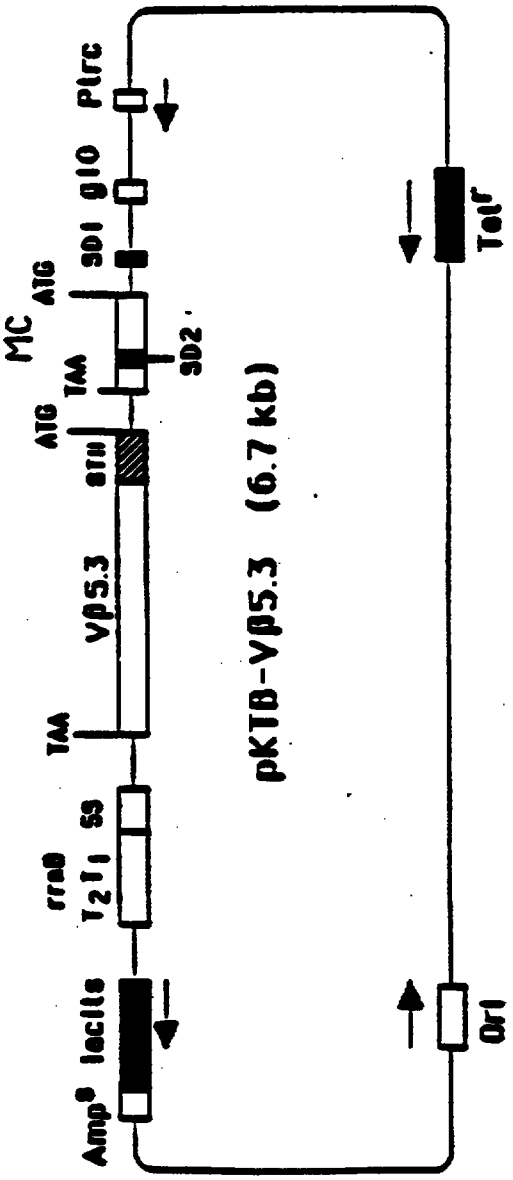
271  AGC AGC     [SEQ ID NO. 25]
     TCG TCG     [SEQ ID NO. 26]
 91  Ser Ser     [SEQ ID NO. 27]
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Figure 2



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T CELL ANTIGEN RECEPTOR V REGION PROTEINS AND METHODS OF PREPARATION THEREOF

INTRODUCTION

The present invention is generally in the fields of immunology and medicine and relates to full length recombinant T cell receptor (TCR) V region proteins, expression vectors for producing commercially useful amounts of recombinant T cell antigen receptor V region proteins, and methods of using full length recombinant T cell receptor V region proteins to diagnose and treat diseases or disorders of the immune system.

BACKGROUND OF THE INVENTION

1. THE T CELL ANTIGEN RECEPTOR

The immune response to foreign antigens is divided into humoral immunity, mediated by B cells, and cellular immunity, mediated by T cells. The receptor molecules used by B cells to recognize antigen are immunoglobulins which recognize conformational epitopes on globular protein. The antigen receptors on T cells are related to immunoglobulins, but bind processed antigen molecules in association with self major histocompatibility complex (MHC) molecules (Dembic et al., 1986, *Immunol. Today*, 7:308).

TCR genes, like immunoglobulin genes, consist of coding regions (or gene segments) which rearrange during T cell ontogeny (Chien et al., 1984, *Nature*, 312:31-35; Hedrick et al., 1984, *Nature*, 308:149-153; Yanagi et al., 1984, *Nature*, 308:145-149). The human T cell receptor ß chain locus has been extensively studied since the cloning of the first cDNA encoding the ß-chain (Yanagi, Y., et al. 1984, *Nature*, 308:145-149; Hedrick et al., 1184, *Nature*, 308:149-152; Siu et al., 1984, *Cell*, 37:393-401). This locus is a gene complex comprising regions encoding known regions or domains of the β chain: the variable (V), diversity (D), and joining (J) domains. These coding regions participate in somatic cell rearrangement with a region encoding the constant (C) domain (Chien et al., 1984, *Nature*, 309:322-326). *In situ* studies identified the location of the TCRß locus on chromosome 7 at 7q35 (Isobe et al., 1985, *Science*, 228:580). By current estimates, this complex spans more than 600 kb and contains 70 to 80 variable region segments (Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Tillinghast et al., 1986, *Science*, 233:879-883; Kimura et al., 1987, *Eur. J. Immunol.*, 17:375-383; Lai et al., 1988, *Nature*, 331:543-546). These V region gene segments are adjacent to two tandemly organized regions, each of which includes a D and a C gene segment followed by a cluster of six or seven J region gene segments (Tunnacliffe et al., 1985, *Nucleic Acids Res.*, 13:6651-6661); Toyonaga et al., 1985, *Proc. Natl. Acad. Sci. USA*, 82:8624-8628). Following successful DNA rearrangement of the V, D, and J coding regions (gene segments), the translated

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ß chain polypeptide pairs with a TCR α chain and can be expressed on the surface of the T cell (reviewed by Kronenberg et al., 1986, *Ann. Rev. Immunol.*, 4:529-591).

The TCR α and δ locus are next to one another on human chromosome 14. TCR δ coding regions are located entirely within the α gene locus (Satyanarayana et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:8166-8170; Chien et al., 1987, *Nature*, 330:722-727; Elliott et al., 1988, *Nature*, 331:627-631). It is estimated that there are a minimum of 45-50 Vα coding regions (Becker et al., 1985, *Nature*, 317:430-434), whereas there are only approximately 10 Vδ coding regions (Chien et al., 1987, *supra*). In peripheral blood, two Vδ coding regions appear to be predominantly expressed, namely, Vδ 1 and Vδ 2.

The γ TCR gene was identified, first in mice (Saito et al., 1984, *Nature*, 309:757-762; Kranz et al., 1985, *Nature*, 313:762-755; Hayday et al., 1985, *Cell*, 40:259-269) and then in humans (Lefranc et al., 1985, *Nature*, 316:464-466; Murre et al., 1985, *Nature*, 316:549-552). The human γ TCR locus appears to consist of between five and ten V, five J and two C coding regions (Dialynas et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:2619).

TCR diversity, and thereby T cell specificity, is derived from several sources (Barth et al., 1985, *Nature*, 316:517-523; Fink et al., 1986, *Nature*, 321:219-225): the multiplicity of germline gene segments (Chien et al., 1984, *Nature*, 309:322-326; Malissen et al., 1984, *Cell*, 37:1101-1110; Gascoigne et al., 1984, *Nature*, 310:387-391; Kavaler et al., 1984, *Nature*, 310:421-423; Siu et al., 1984, *Nature*, 311:344-349; Patten et al., 1984, *Nature*, 312:40-46); combinatorial diversity through the assembly of different V, D, and J coding regions (Siu et al., 1984, *Cell*, 37:393-401; Goverman et al., 1985, *Cell*, 40:859-867); and junctional flexibility, N-region diversity and the use of either multiple D regions or any of the three translational reading frames for Dß segments. TCR diversity does not appear to arise from the somatic hypermutation mechanism observed for immunoglobulins (Barth et al., *supra*). As a result of these mechanisms, TCRs are generated which differ in their amino-terminal, or N-terminal, domains (i.e., are constructed from combinations of V, D, and J coding regions) but are similar elsewhere, including their carboxyl-terminal, or C-terminal domains (which constitute constant regions of TCRs). Accordingly, an extremely large repertoire of TCR molecules is generated.

Study of the structure and diversity of the human TCR ß-chain variable coding regions has led to the grouping of these coding regions into distinct V region subfamilies (Tillinghast et al., 1986, *Science*, 233:879-883; Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Borst et al., 1987, *J. Immunol.*, 139:1952-1959). Each V region subfamily can be characterized by a consensus sequence and contains similar coding regions exhibiting greater than about 75% similarity in the DNA sequence. For instance, sixteen mouse Vß sequences have been categorized into fourteen different subfamilies by a proposed arbitrary, but simple,

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numerical nomenclature for the Vβ gene segments (see Barth et al., 1985, "The Murine T Cell Receptor Employs a Limited Repertoire of Expressed Vß Gene Segments", *Nature*, 316:517-523). According to this nomenclature, members of the same subfamily share the first digit and differ in second; therefore the Vß8.1, Vß8.2 and Vß8.3 are all members of the Vß8 subfamily. A similar system has been proposed for the human coding regions. In humans, the approximately 60 functional Vß coding regions have been grouped into at least 24 families (Toyonaga and Mak, 1988, *Ann. Rev. Immunol.*, 5:585; Wilson et al., 1988, *Immunol. Rev.*, 101:149; and Robinson, M.A., 1991, *J. Immunol.*, 146:4392).

2. T CELLS IN AUTOIMMUNITY

T cells play a pivotal role in the differentiation and regulation of effector mechanisms within the immune system (Paul et al., 1987, *Science*, 195:1293-1300). The co-recognition of antigen and major histocompatibility molecules by a T cell must be specific and precisely regulated, since improper immune regulation fosters autoimmunity. Several laboratories have studied diseases in which there appears to be improper immune regulation, such as autoimmunity and some forms of immunodeficiency, and have implicated T cells in the pathogenesis of such diseases.

Several undesirable immune responses are characterized by a clonal or oligoclonal expansion of a particular TCR composition as, for example, in conditions of malignancy which have resulted in a T cell leukemia or lymphoma. (See, e.g., Jack et al., 1990, *Am. J. Pathol.*, 136:17; Nitta, et al, 1990, *Science*, 249:672; and, Yoshino et al., 1991, *Int. J. Cancer*, 47:654). In T cell leukemias or lymphomas, the TCR acts as a unique tumor marker because it is stably rearranged and presented on the surface of the cell. A particular TCR composition is also implicated in the recipient of an organ graft whose T lymphocytes have TCRs reactive with the MHC antigens of the donor individual, as for instance the donor of a bone marrow graft.

Several groups have reported selective TCR V region expression in certain autoimmune situations. For instance, Grunwald et al., have noted a preferential expression of the Vα2.3 gene product in CD4+ T cells in the bronchoalveolar lavage when compared to peripheral blood lymphocytes of patients with Sarcoidosis (Grunwald, J. et al., 1992, *Eur J. Immunol.*, 22:129). In Kawasaki disease the preferential expansion of Vß2 and Vß8 T cells was noted at the onset of disease (Abe, J., et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066).

Rheumatoid arthritis (RA) has been extensively studied in this regard. Several investigators have noted preferential expansion of certain of T cell subsets, e.g., DerSimonian, et al., 1993, *J. Exp. Med.*, 177:1623 (preferential expansion of Vα12.1-bearing T cells in CD8+ peripheral blood T lymphocytes); Stamenkovic, et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:1179 (synovial membrane-infiltrating T cells grown in IL-2 were oligoclonal by Southern

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blot analysis); Paliard, et al., 1991, *Science*, 253:325 (hypothesizing that superantigen-activated Vß14 T cells, including autoreactive T cells, expand clonally and migrate to the synovial fluid of RA patients); Howell et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:10921 (noting particularly Vß3, 14, and 17 T cell V region gene usage in IL-2R+ cells from synovial fluid of RA patients); Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8534 (oligoclonal T cell V region gene usage in synovial fluid T cells of a single RA individual); International Patent Publication WO 90/06758 (implicating Vß3, 9, and 10 in RA).

Inflammatory bowel disease has also been extensively studied. Several groups have noted expanded T cell population or preferential TCR V region gene usage, e.g., Posnett et al., 1990, *J. Clin. Invest.*, 85:1770; Spencer et al., 1991, *J. Clin. Pathol.*, 44:915; Trejdosiewicz et al., 1991, *Clin. Exp. Immunol.*, 84:440; Van Kerckhove et al., 1992, *J. Exp. Med.*, 175:57. Still others have reported preferential TCR V gene usage in *Mycobacterium leprae* (van Shooten et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:11244; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188.

Multiple sclerosis (MS) is an autoimmune disease characterized by central nervous system mononuclear cell infiltration and demyelination. Although the pathogenesis of MS is unknown, both genetic and environmental factors have been implicated in the disease process. Major elements of the genetic predisposition include an association of disease with particular class II major histocompatibility complex (MHC) haplotypes (Terasaid et al., 1976, *Science*, 1933:1245-1247; Ho et al., 1982, *Immunogenetics*, 15:509-517: Spielman et al., 1982, *Epidemial. Rev.*, 4:45-65; Francis et al., 1986, *Lancet*, 1:211; Elian et al., 1987, *Disease Markers*, 5:89-99 ; Urban et al., 1988, *Cell*, 54:577-592; Vandenbark, et al., 1989, *Nature*, 341:541-544; Howell et al., 1989, *Science*, 246:668-670).

It has been shown that T cells isolated from the cerebral spinal fluid of patients suffering from MS utilize a limited set of TCR V region genes. The demonstration of *in vivo* activated myelin basic protein (MBP)-specific T cells in MS patients implicates MBP-reactive T cells in the pathogenesis of the disease (Wucherpfennig, K.W., et al., 1990, *Science*, 248:1016-1019). Studies using polymerase chain reaction (PCR) amplification of cDNA with TCR Vß primers indicated that MBP-reactive T cell lines use only a limited number of Vß coding regions (Wucherpfennig, K. W., et al., 1990, *supra* (Vß17, and to a lesser extent Vß12, are frequently used in the recognition of the immunodominant region of the human autoantigen MBP); Oksenberg J. R., et al., 1993, *Nature*, 362:68-70 (rearranged Vß5.2 and/or Vß5.3 genes were detected in the brains of patients with a certain HLA phenotype); Kotzin, B. L., 1991, *Proc. Natl. Acad. Sci. USA*; 88:9161-9165 (a bias for use of the ß chain variable region 5.2/5.3, and to a lesser extent Vß 6.1, seen among MBP-specific clones from patients with MS)). The results of

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some studies also imply that there is a limited TCR Vα gene expression in MS brain lesions (Oksenberg, J. R., et al., 1990, *Nature*, 345:344-346).

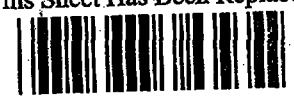
3. APPROACHES TO T CELL MEDIATED THERAPY

In the past, efforts to selectively alter the T cell population of an individual have focussed on vaccination with whole live or attenuated T lymphocytes (for a review of this approach see Cohen, I.R.,1986, *Immunol. Rev.*, 94:5-21) or with peptide vaccines based on selecting a specific peptide portion of the TCR V region. These approaches have been based on the current view that potentially pathogenic T cells preferentially utilize a limited set of V coding regions. The approach developed by Cohen uses live or attenuated T cells as a vaccine to treat or prevent, *inter alia*, experimental autoimmune encephalomyelitis (EAE). However, this approach is limited because it depends on the isolation if clones of disease-specific T cells: it is necessary to treat each patient with his own expanded cells, thus requiring "tailor-made" or individualized therapeutics. (See, Zhang, et al., 1993, *Science*, 261:1451).

Others have suggested the therapeutic use of TCR peptides based on TCR V region involvement (Offner, H., 1991, *Science*, 251:430 (treatment with a peptide based on the TCR Vß8 amino acid sequence reduced severity of disease and accelerated recovery in Lewis rats); Hashim, G.A., et al., 1992, *J. Immunol.*, 149:2803-2809; Offner, H. et al., 1992, *J. Immunol.*, 148:1706-1711; Offner, H., et al., 1991, *J. Immunol.*, 146:4165-417; *but see* Sun, D., 1992, *Cell. Immunol.*, 141:200-210). These peptides are generally short peptides, usually between 8-25 amino acids in length, and rarely more than 30 amino acids in length. For example a 21 amino acid peptide present on the TCR chain associated with EAE has been used to prevent EAE in Lewis rats (Vandenbark A.A. et al., 1989, *Nature*, 341:541-544; Vandenbark, A.A., International Patent Publication WO 91/01133; see also, Janeway, C.A., 1989, *Nature*, 341:482-483). Others have reported that even shorter peptides of 8 or 11 amino acids, corresponding to portions of the ß chain VDJ region or the Jα region proved effective as vaccines for either preventing or reducing the severity of EAE in a rat model (Howell, M.D. et al., 1989, *Science*, 246:668-671; Howell, M.D. et al., International Patent Publication WO 92/12996).

Peptides derived from TCR proteins have commonly been chosen as candidates for autoimmune therapeutics because of the ease of peptide synthesis and because no method has been previously available for the production of longer TCR V region proteins in useful quantities and having significant biological activity. More importantly, the major drawback of small synthetic peptides is that their use requires a prediction of the particular sequence that would be effective in modulating an immune response. Predictions must be made as to the antigenic or immunogenic properties of the proposed peptides, as for example their occurrence in the hypervariable or complementarity-determining region (CDR) of a V region. Additionally,

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the size of the proposed peptide derived from a particular TCR must be calculated to maintain the proper epitope structure to prime suppressor T cell activity or to elicit release of antibodies directed against the subpopulation of T cells bearing that particular TCR (to which the peptide corresponds). Such peptide design requires substantial time and experimentation; and in many instances where human therapies are concerned, the necessary experimentation may not be performed, for humane or ethical reasons.

In addition, in order to obtain a specific antigenic response by a regulatory T cell, the peptide must be bound to an individual's MHC molecules. These MHC antigens present on target cells have polymorphic regions wherein specific alleles are associated with particular individuals. For the most part, the individual will have two haplotypes, meaning that there will be two different alleles of a particular MHC antigen from the same locus. Not all peptides will have the capacity to bind to MHC antigens, nor will all MHC antigens bind to any single peptide, since there are multiple MHC antigens for each haplotype. Therefore, the suitability of such a peptide approach to T cell-mediated therapeutics must be determined on the haplotype of each individual, requiring tailoring of peptides to the individual patient.

4. PRODUCTION OF SOLUBLE T CELL ANTIGEN RECEPTOR

Production of large amounts of soluble TCR proteins by recombinant techniques has been problematic for several reasons. A major obstacle to overcome in the production of soluble T cell antigen receptor is the secretion in the absence of the transmembrane region (Traunecker, A. et al., 1989, *Immunol. Today*, 10:29-32). One investigator was able to show secretion of a full length monomeric ß chain of the murine TCR including the V, D, and J regions followed by a truncated form of the Cß region. The methodology took advantage of the homology between the carboxyl-terminal end of the immunoglobulin light chain and the sequence around Cys 241 in the TCR ß chain. The resulting construct utilized the chimeric gene encoding the k-chain leader peptide sequence from MPC-11 and a rearranged VDJ exon. The resulting construct was expressed in mammalian cells and was detectable in the cell supernatant. The method did not suggest, however, a means of obtaining a full length V region protein in the absence of extraneous protein sequences, since secretion was dependant on the presence of the truncated Cß exon (Gasgoigne, N., 1990, *J. Biol. Chem.*, 265(16):9296-9301).

Another group attempted to achieve the expression of a soluble form of the TCR ß chain in biochemically significant quantities by the expression of a recombinant TCR heterodimer anchored by a phosphatidyl inositol glycan linkage. The method produced small (0.5mg/harvest) amounts of a full TCR chain which necessitated the cleavage from the surface of the transfected cells by a phosphatidyl inositol-specific phospholipase C (Lin, A.Y., 1990, *Science*, 249:677-679).

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Others have produced chimeric molecules by shuffling the variable ~~and constant~~ domains of the murine TCR with the constant region of an immunoglobulin κ light chain. The investigators were able to achieve small amounts of a secreted soluble form of a heterodimeric αß TCR (Gregoire, C., et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8077-8081). Gregoire et al. show a murine chimera consisting of the Cα and Vα genes of the KB5-C2 joined to the C region of the κ light chain of the S105 monoclonal antibody. Also shown is a VßCßCκ chimera. Both are transfected into a mammalian B cell myeloma that does not express native immunoglobulin heavy or light chains. (See also, Weber, S., et al., 1992, *Nature*, 356:793-795).

Novotny et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8646-8650, demonstrate a method for producing soluble TCRs with antigen-combining properties. A synthetic oligo linker was cloned onto the C terminus of the Vß and the N terminus of the Vα region resulting in a single-chain VßVα. The resultant expression vector encodes the pelB leader followed immediately by in frame Vß, the linker, and the Vα segments. The expression of this construct is under the control of the lacZ promoter and is therefore IPTG-inducible.

Recently, one group has shown the secretion of a small amount of a T cell receptor V region fused to a carboxyl-terminal poyhistidine "tail". The plasmids contain the pelB leader and are under the control of a chemically inducible promoter (Ward, E.S., 1991, *Scand. J. Immunol.*, 34:215-220). The work fails, however, to describe how to obtain a T cell antigen receptor V region protein in the absence of a fusion partner, or how to obtain commercially useful amounts of the receptor V region.

There is a great need, therefore, for agents and pharmaceutical compositions which have the properties of specificity for the targeted autoimmune response, predictability in their selection, and convenience and reproducibility of preparation.

SUMMARY OF THE INVENTION

The present invention addresses the above-mentioned problems by providing for the first time the successful production of a universal agent for treating a given T cell-mediated disease in which T cell subsets are implicated. In particular, the invention provides useful quantities of full length TCR V region proteins that may be naturally processed, providing for multiple active fragments upon *in vivo* processing, and presented by antigen-presenting cells of each individual, thereby eliminating the need for "tailor-made" therapeutic agents. Also provided is a class of recombinant expression vectors for the production of TCR V region proteins in bacteria. The recombinant TCR V region proteins of the instant invention are useful in the treatment, prevention or suppression of an immune-related disorder. In another embodiment, the TCR molecules can be combined in an admixture with a pharmaceutically acceptable carrier for use as a therapeutic composition. In another embodiment, the claimed

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TCR V region proteins are useful to generate anti-TCR antibodies. Alternatively, the TCR V region proteins can be used diagnostically to characterize a particular immune-related disorder. The TCR V region proteins of the instant invention can be used as diagnostic probes based their ability to bind with autoantibodies. It is an advantage of the present invention that the T cell antigen receptor V region proteins are expressed in the absence of fusion proteins or affinity tails. It is a further advantage of the present invention that the TCR V region proteins are produced in commercially acceptable amounts that enable the preparation of compositions which are therapeutically effective in treating T cell-mediated autoimmune disease.

The invention further provides for an expression vector for the production of a pure full length TCR V region molecule. The expression vectors of the invention include as an essential element a coding region for a T cell antigen receptor V region. The TCR coding region of the invention is any nucleic acid molecule encoding a full length TCR V region protein. In a preferred embodiment, the TCR coding region includes the structural coding sequence for the Vß5.3 region.

The expression vectors described herein provide a means for the inducible expression of TCR V region proteins. In preferred embodiments, the T cell antigen receptor V region coding sequence is under the transcriptional control of an inducible bacterial promoter. According to the present invention, the inducible bacterial promoter is subject to either chemical or thermal regulation. Most preferably, the inducible bacterial promoter is subject to thermal induction.

In one embodiment, the expression vector of the instant invention includes a regulatory gene coding for a protein capable of binding to the promoter and thereby repressing transcription from the inducible promoter. In a preferred embodiment, the promoter is derived from the *E. coli lac* operon and the regulatory gene comprises a functional mutation of the *E. coli lacI* repressor gene. This mutant *lacI* gene (*lacI*ts) codes for a temperature-sensitive repressor protein, and utilizing this *lacI*ts gene permits thermal induction of the promoter. In a preferred embodiment, the inducible bacterial promoter is a *trc* promoter, and the thermal induction is provided by the *lacI*ts regulatory gene.

In one embodiment, the regulatory gene is located on a cotransformed vector. In another embodiment, the regulatory gene is located in the bacterial host cell chromosome. In a preferred embodiment, the regulatory gene is located in the recombinant expression vector. In a further preferred embodiment, the transcription direction of the mutant *lacI* gene is in the same orientation as the T cell antigen receptor structural gene to be expressed.

The recombinant expression vectors of the present invention may advantageously include a coding sequence for a leader or signal peptide. Such a leader sequence, fused in frame and 5' to the TCR Vregion coding region to be expressed, directs the synthesis of a TCR V

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region protein having an N-terminal leader peptide which in turn directs secretion of the recombinant protein across the host cell membrane. Other embodiments of the present invention do not utilize a leader sequence, and the desired V region product is isolated from the cells, e.g., by urea extraction.

In a preferred embodiment, the recombinant expression vectors of the present invention comprise, in the 5' to 3' direction: a *trc* promoter, a ribosome binding site, an optional leader sequence, a coding region for a full length T cell antigen receptor V region, a stop codon, one or more transcription terminators, a *lacI*ts regulatory gene such that the transcription direction is the same as that of the coding region for the T cell antigen receptor V region, a bacterial origin of replication, and a tetracycline resistance gene.

This invention also provides methods for producing TCR V region proteins at significantly increased levels than previously reported.

The present invention provides compositions and methods for treating a patient having an immune-related disorder. Such treatment comprises administering to a subject in need of such treatment, a composition in accordance with the present invention, in an amount and for a period of time suitable to regulate said immune disorder. It is an advantage of the present invention that the compositions of the invention allow for treatment of an immune related disorder without the need to select a particular peptide fragment and without tailoring the particular composition to the particular individual to be treated.

The invention also relates to the use of a full length T cell antigen receptor V region protein in therapy for an autoimmune disease or disorder. In a specific embodiment, the invention relates to the use of a full length Vß5.3 protein in therapy. In another embodiment, the invention provides for the use of a full length T cell antigen receptor V region protein in the manufacture of a medicament for treating an autoimmune disease or disorder. Therefore, in one embodiment, the invention enables the use of a full length Vß5.3 protein in the manufacture of a medicament for the treatment of an immune related disease or disorder such as MS.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. The complete nucleotide sequence [SEQ ID NO. 25 and SEQ ID NO. 26] and amino acid sequence [SEQ ID NO. 27] of the full length Vß5.3 protein of the invention.

Figure 2. Plasmid map of pKTB-Vß5.3. This plasmid contains Ptrc, the *trc* promoter; g10, the g10 leader gene segment from bacteriophage T7; SD1, ribosome binding site 1; MC, a mini-cistron encoding ribosome binding site 2 (SD2) AAG GAG (see SEQ ID NO. 28 for complete mini-cistron nucleotide sequence) and the peptide Met Tyr Arg Leu Asn Lys Glu Glu (SEQ ID NO. 29); STII, the STII leader sequence; Vß5.3, the Vß5.3 coding region; T1T2,

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transcription terminators of the *rrnB* operon; the *lacI*ts gene encoding the temperature-sensitive *lac* repressor; Ori, the origin of replication and Tet^r, the tetracycline resistance gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes recombinant expression vectors and methods for the efficient production of large quantities of a T cell antigen receptor V region protein and the use thereof, including the diagnosis, treatment, prevention or suppression of immune-related diseases or disorders.

A series of vectors has been developed for the production of enhanced levels of a full length TCR V region protein. Additionally, a method is provided for producing commercially useful amounts of a full length recombinant TCR V region protein using an expression vector of the instant invention. The methods and expression vectors of the instant invention are unique because they provide for the production of a full length T cell antigen receptor V region in the absence of extraneous protein sequences or fusion partners. As detailed in the Background of the Invention, *supra*, T cell antigen receptor V regions have, in the past, been produced only as part of a larger protein, and at very low levels. These extraneous proteins may cause undesirable immunogenicity when used *in vivo*, and they are frequently cited by regulatory authorities as impermissible impurities. Previously purified V region proteins included other regions of the TCR, such as the C region, fused to the V region. Others constructed expression systems that provided for a TCR V region as a chimeric protein, e.g., a V region fused to a portion of an immunoglobulin molecule.

The expression vectors and methods of the instant invention further provide for enhanced levels of expression of a T cell antigen receptor V region protein. A primary consideration in the production of a protein that has commercial utility, for example, in research, diagnostics and therapeutics, is the manufacture of commercially useful amounts of a protein. The present invention therefore provides for the production of enhanced levels of TCR V region proteins. The present invention makes possible the production of V region protein in large amounts, e.g., from 1 and 500 mg/liter of bacterial culture, or more. Preferably, the production level of the TCR V region protein is at least 100 mg/liter. In a most preferred embodiment, the instant invention provides for the production of at least 300 mg of Vβ protein per liter of bacterial cell culture.

The methods and expression vectors of the instant invention provide for the production of a TCR V region protein in the absence of extraneous protein sequences. The invention therefore eliminates several steps associated with obtaining a purified TCR V region protein if prepared according to the prior art. In one embodiment, the present invention provides for

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increased expression of V region proteins by thermal induction, which avoids the necessity of eliminating chemical inducers, such as IPTG.

Because TCR V region proteins are produced according to the invention in the absence of extraneous proteins, the invention provides for simplified production of relatively pure product. The invention eliminates additional processing steps, such as the introduction of enzymes or chemical reagents into the system to obtain a protein free of any fused non-TCR peptide segments or affinity tails. Not only are such manipulations likely to reduce the amount of V region product recovered, they also introduce elements which must be eliminated, i.e., by additional purification steps.

1. T CELL ANTIGEN RECEPTOR V REGION

The T cell antigen receptor V region protein of the instant invention is any full length T cell antigen receptor V region. A full length TCR V region protein is encoded by a coding region for a T cell antigen receptor V region. The TCRV region coding region is a nucleotide sequence encoding a full length T cell antigen receptor V region protein. Within the scope of the present invention, V region includes the variable region of the T cell antigen receptor. In its broadest aspects, the teachings of the present invention may be applied to obtain enhanced expression of TCR proteins including the V region and, additionally, all or part of the D and/or J regions.

TCR V region protein is produced in the absence of extraneous protein sequences, such as, for instance, other TCR regions, such as the constant region, the transmembrane region or the cytoplasmic region. Further, the TCR V region protein of the invention is produced in the absence of non-TCR fusion partners, such as affinity tails. Further, the TCR V region protein of the invention is not produced as a part of a larger chimeric protein including portions of other protein molecules, such as an immunoglobulin protein.

Recognizing that the DNA sequence encoding one of the complementarity-determining regions (CDRs) of a T cell receptor's V region may actually span the known juncture between the V and D coding regions (as in the case of the CDR3 of Vβ 5.3), as used herein, a "full length" V region coding region may include the entire coding sequence of such a CDR that spans the V-D juncture, the portion of such a CDR that only resides in the known V region, or none of the sequence coding for such a CDR. Furthermore, the full length T cell antigen receptor V region protein of the present invention can be any of the T cell antigen receptor V regions and is not limited to only Vβ regions (i.e., V regions of the TCR β-chain). Therefore, the T cell antigen receptor V region protein can be a variable region of the α subunit, the β subunit, the δ subunit, or the γ subunit.

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In a preferred embodiment, the TCR V region protein of the instant invention is any T cell antigen receptor V region for which a full length coding sequence is available. Methods of obtaining the full length coding sequences of the instant invention are well known in the art. Human V region domain DNA sequences can be isolated in accordance with well known procedures from a variety of human cells. Preferably, the cells are peripheral blood T lymphocytes.

The DNA sequences of the present invention may be prepared in various ways. It is preferred that the DNA sequences of the T cell antigen receptor V regions be prepared by polymerase chain reaction (PCR) using DNA sequences coding for the intact TCR as templates. A human Vβ region can be prepared, for instance, via amplification of cDNA prepared from human peripheral blood lymphocytes stimulated by an anti-CD3 antibody, following the procedure of Choi et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:8941-8945.

In one aspect of the present invention, the coding region for a full length T cell antigen receptor V region is obtained from any clone containing the full length sequence. For example, several TCR V region sequences and cDNA clones containing the full length coding regions are described in Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602.

Preferred TCR V region proteins of the instant invention are those implicated in an immune-related disease or disorder, particularly, e.g., Vβ 8.1 (Kawasaki disease; see, Abe et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066), Vβ 6.1 (leprosy; see, Wang, et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188), Vβ5.1 (Lyme disease; see, Lahesma et al., 1993, *J. Immunol.*, 150:4125), Vβ5.2, or Vβ5.3, or Vβ6.1 (multiple sclerosis; see Kotzin, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:9161), Vβ2.1, or Vβ3.1 (rheumatoid arthritis; see Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8534). (See also, Hafler, D.A. et al., 1988, *J. Exp. Med.*, 167:1313; and Mantgazza R. et. al., 1990, *Autoimmunity*, 3:431).

The TCR expressed by a T cell clone in an autoimmune disease or a T cell clone responding to a particular autoantigen can be identified using TCR-specific antibodies, for example polyclonal, monoclonal or chimeric antibodies that are specific for a TCR variable segment, in conjunction with an antibody labelling method, including fluorescence microscopy, flow cytometry, immunocytochemistry, or other techniques known in the art. Such antibodies have been described for a number of TCR α and/or β chain V regions (see, for example, International Patent Publication WO 90/06758).

Alternatively, the DNA or mRNA of the T cell clone can be probed directly, or after amplification by PCR (Synha et al., 1988, *Science*, 239:1026; Saiki et al.,1986, *Nature*, 324:163), and specific hybridization with nucleic acid probes for the various TCR gene families,

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using hybridization methods well known in the art. The TCR sequence, or a part thereof, can then be obtained directly from the amplified, rearranged DNA or mRNA.

Expression of a particular TCR can also be identified by determining the nucleic acid sequence encoding at least part of the TCR, for example, after cloning the TCR V gene, or by determining the amino acid sequence of at least part of a TCR protein. It will be apparent that any of the above-mentioned approaches, or additional approaches known to one of skill in the art, will result in the identification of the TCR expressed on a T cell or clone or line of T cells.

Examples of molecular approaches used to correlate TCR gene expression with disease include:

(1) producing and analyzing cDNA libraries obtained from the disease-related T cells obtained from one or more subjects having the disease, to determine the presence of frequently used or 'dominant' TCR genes;

(2) Southern analysis of disease samples to determine whether specific genetic polymorphism (e.g., RFLPs) or oligoclonal TCR rearrangements exist;

(3) analysis of disease samples by cDNA synthesis, PCR amplification, and slot blot hybridization procedures; and

(4) *in situ* nucleic acid hybridization of TCR probes to T cells without prior culture of these cells.

Preferred TCR V region proteins include Vβ8.1, Vβ5.2, or Vβ5.3. Double-stranded cDNA encoding Vβ5.3 and the amino acid sequence for Vβ5.3 are shown in Figure 1. (See, SEQ ID NOS. 25, 26 and 27.)

A T cell antigen receptor V region clone can be introduced into the expression vectors of the instant invention by methods known to those skilled in the art. For example, from a cDNA clone, the V region is PCR synthesized incorporating an initiating ATG at the 5' terminus. Any convenient restriction site can be synthesized which incorporates an initiating codon and reconstitutes the 5' coding region of the V region. In a preferred embodiment, the restriction site is an *Nco*I site. A stop codon and an appropriate restriction site are engineered at the 3' end of the V region coding sequence so that it can be subcloned into the vector.

It should be understood that the methodology of the present invention can be used to prepare full length TCR V regions derived from animal species other than human.

2. EXPRESSION VECTORS

A recombinant expression vector within the scope of the present invention is a plasmid which contains exogenous DNA in the form of a nucleic acid coding TCR V region to be expressed. The recombinant expression vector of the present invention is introduced into a

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bacterial host cell for example by transformation, and transformants are isolated and cloned which express the desired V region protein.

The present invention concerns expression vectors containing the DNA sequence coding for the full length TCR V region protein. Preferably, the TCR V region proteins are those T cell antigen receptor proteins specifically described herein. An expression vector within the scope of the present invention is a plasmid which contains the TCR V region gene to be expressed under the transcriptional and translational control of regulatory elements, such as for instance the promoter, ribosomal binding site and transcription terminator. In addition it contains an origin of replication for stable replication in bacteria. It also preferably includes a gene coding for resistance to an antibiotic to facilitate selection of plasmid-bearing transformant cells. Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell.

Expression vectors useful in the present invention are often in the form of plasmids. Plasmids refer to circular double-stranded DNA sequences which are not integrated in the chromosome. The expression vectors of the present invention may also include other DNA sequences, for example leader or signal sequences which provide for targeting or stability of the expression product, and/or regulatory sequences which allow modulation of gene expression, for example in response to a shift in temperature or a change in composition of the growth medium.

The expression vectors contain one or more control sequences operably linked to the DNA sequences coding for the TCR V region. The term "operably linked" within the scope of the present invention means that one nucleic acid sequence ,which has a regulatory function for gene expression or encodes a functional protein domain, is covalently linked, either 5' or 3', to a second nucleic acid sequence encoding a protein, so that the regulatory function is exerted over the second nucleic acid sequence or the encoded functional domain will be fused in frame to the protein encoded by the second nucleic acid. Such regulatory functions for gene expression typically include initiation of transcription, termination of transcription, and mRNA transcript processing. Nucleic acid sequences that exert such regulatory functions over gene expression include promoters of transcription, transcription terminators, and mRNA processing signals. Functional domains that may be "operably linked" to another protein include, for example, leader or signal peptides which permit transport of proteins across cell membranes.

In one embodiment, the promoter may be any inducible promoter. In a preferred embodiment, the promoter is one that is regulated by the presence or absence of certain media components. One skilled in the art will recognize that several bacterial promoters subject to the control of the presence or absence of certain media components can be used in connection with the expression vectors of the instant invention. It is preferred that the promoter be a strong

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bacterial promoter such as the *lac* promoter, the *tac* promoter, and the *trc* promoter. Therefore, in one embodiment, a promoter for a TCR V region coding sequence in a vector of this invention is subject to regulation by the presence or absence of isopropyl-ß-D-thiogalactopyranoside (IPTG), a gratuitous chemical inducer molecule of the *lac*, *tac*, and *trc* promoters. In preferred aspects, the IPTG-inducible promoter is the *lac* promoter or the *tac* promoter. In a more preferred embodiment, the IPTG-inducible promoter is the *trc* promoter (Brosius et al., 1985, *J. Biol. Chem.*, 260:3539-3541) which is similar to the *tac* promotor derived by fusing the -10 region of the *lac* UV5 promoter with the -35 region of the tryptophan operon (de Boer et al., 1983, *Proc. Natl. Acad. Sci. USA*, 80:21-25; Amann et al., 1983, *Gene*, 25:167-178.) The *trc* promoter is identical to the *tac* promoter with the exception of a single base pair change in the promoter (Brosius et al., (1985) *supra*).

In a further embodiment, the expression vector of the instant invention is modified to allow for the secreted expression of the full length TCR V region protein of the invention by a temperature shift. According to this embodiment, the expression vector allows for the expression of TCR V region protein in the absence of chemical inducers, which obviates the need to use such elements as IPTG and bacterial host strains containing the *lacI* or the *lacI*^{q} genes. Preferred embodiments of the expression vectors of the instant invention include a regulatory gene which allows for the thermal induction of protein expression. The regulatory gene sequence most preferably is the *lacI*ts gene, which encodes a temperature-sensitive *lac* repressor.

In a further preferred embodiment, the *lacI*ts gene is located proximal to and downstream of the transcription terminator, and its transcription direction is the same as that of the V region coding sequence.

In addition, the expression plasmids of this invention preferably contain one or more selectable genetic marker(s). Preferably, the selectable genetic marker confers resistance to a antibiotic, such as ampicillin, kanamycin or tetracycline. Most preferably, the selectable marker is the tetracycline resistance gene.

Additionally, the vector includes a bacterial origin of replication. In a preferred embodiment, the origin of replication is derived from pBR322. The expression vector also contains a ribosome binding site and the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118.)

In a preferred embodiment, an expression vector of the instant invention contains as essential elements in the 5' to 3' direction: a DNA sequence which contains a promoter and an operator; a DNA sequence which contains a ribosomal binding site for rendering the mRNA of the desired gene capable of binding to ribosomes within the host cell; an ATG initiation codon

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or a DNA sequence which is converted into an ATG initiation codon upon insertion of the desired gene into the vector; a restriction site for the insertion of the desired gene in frame with the ATG initiation codon or a multiple cloning site containing several restriction sites for the insertion of the appropriate gene or coding region to be expressed; a nucleic acid sequence encoding the T cell antigen receptor V region to be expressed; and a transcription terminator which serves to efficiently terminate transcription. The vector also contains a DNA sequence which contains an origin of replication from a bacterial plasmid capable of autonomous replication in the host cell and a DNA sequence which contains a gene associated with a selectable or identifiable phenotypic trait which is manifested when the vector is present in the host. The expression vector may optionally include a signal or leader coding sequence operably linked, i.e., fused in frame, to the 5' end of the nucleic acid sequence encoding the TCR V region so that translation of the leader sequence-TCR V region sequence results in a leader peptide-TCR V region fusion protein in which the leader peptide at the amino terminus of the fusion protein directs secretion of the TCR V region protein across a host cell membrane. In certain preferred embodiments, the expression vector contains a regulatory gene which allows for the expression of the secreted protein by a temperature shift.

In a more preferred embodiment, the expression vector contains the following essential elements in 5' to 3' direction: The *trc* promoter which is composed of the -10 region of the *lac* UV5 promoter fused with the -35 region of the tryptophan operon (de Boer et al., *supra*; Amann et al. (1983) *supra*); a strong ribosome binding site; the TCR V region coding region to be expressed; the two transcription terminators from the *rrnB* ribosomal RNA operon; the *lacI*ts gene coding for the temperature-sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. An example of such a preferred embodiment, the expression vector pKBi-Vβ5.3 was deposited under the Budapest Treaty with the American Type Culture Collection (ATCC) under accession no. 69739 on January 6, 1995.

Expression vectors of the invention may contain a leader sequence encoding a signal peptide operably linked 5' of the TCR V region coding region. On expression, amino-terminal signal peptide will direct secretion of the TCR V region protein across the host cell membrane, into the periplasm or the culture medium. One skilled in the art will recognize that many leader sequences may be used in connection with the expression vector of the instant invention. One suitable leader sequence is the pelB signal sequence from *E. carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383). Another suitable leader sequence is the heat stable enterotoxin II (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732).

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In one embodiment, the expression system contains the STII leader sequence, and the coding region for a V region is PCR synthesized to contain an appropriate restriction site at the 3' end and an engineered *Mlu*I site at the 5' end so that it can conveniently link to the *Mlu*I site incorporated into the 3' end of the STII leader. Therefore, in a further preferred embodiment, the expression vector comprises in the 5' to the 3' direction: the *trc* promoter which is composed of the -10 region of the *lac* UV5 promoter fused with the -35 region of the tryptophan operon; a strong ribosome binding site; the STII leader; the coding region for the T cell antigen receptor V region to be expressed; the transcription terminators from the *rrnB* operon; the *lacI*ts gene coding for the temperature sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. In a most preferred embodiment, the expression vector is pKB-Vβ5.3.

The expression vectors of the present invention may be constructed using standard techniques known in the art. (See, e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

The present invention additionally concerns host cells containing an expression vector which contains a DNA sequence coding for a full length TCR V region. Additionally, preferred host cells contain an expression vector comprising one or more control DNA sequences capable of directing the replication and or the expression of a DNA sequence coding for a full length T cell antigen receptor V region protein. Suitable host cells include prokaryotic host cells; and in preferred aspects, the prokaryotic host cell is a bacterial host cell, most preferably an *E. coli* cell.

One skilled in the art will understand from the teaching of the instant application that the particular expression vector used will influence the choice of host strains selected for the production of the full length TCR V region proteins. For instance, in the expression vectors subject to chemical induction within the scope of the present invention, bacterial host strains which harbor the *lacI* or *lacI*^{q} gene, which regulate the *trc* promoter, are especially useful. In expression vectors within the scope of the present invention that contain a regulatory gene such as the *lacI*ts gene, which provides for a temperature-sensitive repressor, bacterial host strains that do not harbor the wild type *lacI* or *lacI*^{q} genes are preferred. A particularly preferred host cell is LJ24.

In certain embodiments, the expression vector of the instant invention does not contain a leader sequence. In certain preferred embodiments the bacterial host strain is *E. coli* BL21, or more preferably a protease deficient *E. coli* strain, such as SG21163, SG22094, or SG21173.

The expression vectors of the present invention may be introduced into the host cells by various methods known in the art which may vary depending on the type of cellular host. For example, calcium chloride treatment or electroporation are commonly utilized for prokaryotic

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cells. (See, generally, Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

Once the expression vector has been introduced into an appropriate host cell, the host cell may be cultured under conditions permitting expression of large amounts of TCR V region.

Host cells containing an expression vector which contains a DNA sequence coding for a TCR V region protein may be identified by any appropriate means. In the following examples, for instance, production of V region protein is detected immunologically using an immunoassay. Alternatively, the host cell containing the expression vector of the instant invention may be identified by assessing the level of transcription of TCR V region DNA by measuring TCR-specific V region mRNA transcripts in the host cell (e.g., by Northern blot analysis). In yet another method, the host cell containing the expression vectors of the instant invention can be identified by DNA-DNA hybridization using probes complementary to the TCR V region coding sequence of the vector.

The DNA sequences of the present invention, expression vectors or DNA molecules may be determined by various methods known in the art. For example the dideoxy chain termination method as described in Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA.*, 74:5463-5467.

Following the examples provided herein and utilizing one or more of the detection methods described above, a person skilled in the art may, without undue experimentation, readily select the combinations of expression vectors and host cells that provide optimal expression of TCR V region proteins. By combining a particular host strain with a particular expression vector of this invention, it is possible to obtain relatively high level expression of full length T cell receptor protein. Preferably, the level of expression of full length TCR V region protein is at least 300 mg per liter of culture. Such levels of expression can be obtained, for example, by using a preferred embodiment in which *E. coli* SG21173 is the bacterial host strain and pKBi-Vβ5.3 is the expression vector (see examples, *infra*). Such combinations of host and expression vectors of this invention permit the production of significant quantities of full length T cell receptor protein at a lower cost than previously available.

Once expressed, the proteins of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity chromatography, column chromatography, gel electrophoresis and the like. (See. generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982).)

In a preferred embodiment, the T cell antigen receptor V region is soluble. By this it is meant that the T cell antigen receptor V region is soluble in an aqueous system. A "soluble" protein is generally characterized by its failure to sediment at room temperature under high G-

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force centrifugation (100,000 x g) out of an aqueous buffer. Solubility is particularly important in providing simplicity in purification of the TCR V region protein from the producing cell. Aqueous buffers used in protein chemistry typically have a buffering compound,for maintaining a particular pH (typically within a physiological range of about pH 5-9), provide a particular ionic strength (typically between about 10 mM and about 500 mM), and often include a protease inhibitor and a mild detergent. Examples of standard buffers are phosphate buffered saline, Tris-buffered saline or any of a number of buffers used in protein isolation. (See, generally, *Methods in Enzymology*; Mahler and Cordes Biological Chemistry (2d Ed.), Harper and Row, New York (1966).)

3. USE OF T CELL ANTIGEN RECEPTOR V REGION PROTEINS

The present invention provides for the first time the means for efficiently and economically producing diagnostically, therapeutically and investigationally useful quantities of a TCR V region protein that is not a fusion protein or linked to an extraneous sequence, such as an affinity tail.

A. Investigational use and diagnostic use

The TCR V region proteins of the present invention are useful in structural and functional studies on the T cell antigen receptor (TCR). Besides serving as substrates or binding domains for specific forms of TCRs, these proteins may serve as tools for conformational studies, e.g., by approximating the native conformation of various portions of the T cell receptor.

The T cell V region proteins can also be used as diagnostic probes. In one specific embodiment, the TCR V region proteins of the present invention may be used in determining the amount or the presence of a certain TCR V region subfamily in a biological sample. One such assay is described in Rittershaus C.W., International Patent Publication WO92/08981, entitled "Therapeutic and Diagnostic Methods Using Total Leukocyte Surface Antigens". As such, the present invention provides methods for diagnosing an immune-related disease, such as multiple sclerosis (MS), by detecting the specific subset of T cells associated with the disease.

In another embodiment, the present invention can be used in the generation of significant quantities of T cell antigen receptor V region proteins for use in antibody production. The antibodies can be prepared by using any of a number of techniques well known in the art. For producing a monoclonal antibody (MAb), any method which provides for the production of antibody molecules by continuous cell lines in culture may be used. These methods include, but are not limited to, the hybridoma techniques originally described by Kohler and Milstein, 1975, *Nature*, 256:495-497); the more recent human B cell hybridoma technique (Kozbor et al., 1983, *Immunology Today*, 4:72); the EBV technique (Cole et al., Monoclonal Antibodies and Cancer

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Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)); and trauma techniques. For a review of antibody production methods see: Hartlaw, E., et al., Antibodies: A laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

The TCR Vregion proteins of the invention can be used as diagnostic probes based on their ability to react with autoantibodies present in a biological sample.

The present invention also contemplates labeled derivatives of the TCR V region proteins which can be detected by appropriate monitors, such as scintillation and Geiger counters, or radiographic film. Such derivatives include, for example ^{125}I, ^{131}I, ^{14}C, ^{35}S, ^{3}H, ^{112}In, ^{99}M, Tc and the like, for *in vitro* and *in vivo* diagnostic purposes.

B. Therapeutic use

As mentioned above, the present invention is useful in the therapy of immune-related disease. The term "immune-related disease" as used herein refers to a disease in which the immune system is involved in the pathogenesis of the disease, or in which appropriate stimulation of the immune system can result in protection from the disease. Relevant diseases include, but are not limited to, autoimmune diseases, neoplastic diseases, infectious diseases, hypersensitivity, transplantation, graft-versus-host disease, and degenerative nervous system diseases. Autoimmune diseases include, but are not limited to, arthritis, such as rheumatoid arthritis, type I diabetes, juvenile diabetes, multiple sclerosis, autoimmune thyroiditis (Hashimoto's thyroiditis), myasthenia gravis, systemic lupus erythematosus (SLE), Sjögren's syndrome, Graves disease, Addison's disease, Goodpasture's syndrome, scleroderma, dermatomyositis, myxedema, polymyositis, pernicious anemia, inflammatory bowel disease including Crohn's disease and autoimmune atrophic gastritis, and autoimmune hemolytic anemia. Neoplastic diseases include, but are not limited to, lymphoproliferative diseases such as leukemias, lymphomas, Non-Hodgkin's lymphoma, and Hodgkin's lymphoma, and cancers such as cancer of the breast, colon, lung, liver, pancreas, melanoma, renal carcinoma, etc. Infectious diseases include but are not limited to viral infections caused by viruses such as HIV, HSV, EBV, CMB, Influenza, Hepatitis A, B, or C; fungal infections such as those caused by the yeast genus *Candida*; parasitic infections such as those caused by schistosomes, filaria, nematodes, trichinosis or protozoa such as trypanosomes causing sleeping sickness, plasmodium causing malaria or leishmania causing leishmaniasis; and bacterial infections such as those caused by mycobacterium, corynebacterium, or staphylococcus. Hypersensitivity diseases include but are not limited to Type I hypersensitivities such as contact with allergens that lead to allergies, Type II hypersensitivities such as those present in Goodpasture's syndrome, and myasthenia gravis.

Therapeutic use of the TCR V region proteins of the invention is premised upon the correlation between a specific immune-related disease and the preferential expression of a

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particular T cell antigen receptor V region protein or the expanded usage of a particular sequence encoding a TCR V region protein. Though the present invention should not be limited by any particular scientific theory, the TCR V regions are useful in part because they may be used to regulate the immune response in an individual. Specifically, where the presence or abnormally high expression of a particular V region correlates with particular immune-related disorder, one can treat the individual by neutralizing the T cells carrying that particular V region protein. Further, introduction of the TCR V region protein may affect immunosuppressive functions by stimulating regulatory T cells which will specifically interact with T cells bearing a targeted V region. It is also likely that the TCR V region protein may elicit an antibody response that will specifically interact with the targeted V region on T cells and in turn modulate the immune response.

The full length T cell antigen receptor V region proteins of the instant invention provide distinct advantages in the treatment of an immune related disease. Prior to the instant invention, therapeutic options for treatment of immune related disease with specific T cell involvement included: (a) no treatment with possible spontaneous resolution; (b) treatment with peptides corresponding to distinct segments of the T cell antigen receptor V region; (c) nonspecific treatments such as steroids or non-steroidal anti-inflammatory agents. However, none of these afforded the particular advantage of the present invention. The therapeutic methods of the instant invention have the distinct advantage over the prior art methods of treating an immune related disease in that the methods of the present invention target only the particular T cell subset expressing a particular TCR V region and can be used across a diverse range of affected individuals, since the invention provides for the elimination of the need to select a particular peptide based on the MHC haplotype of a particular individual. The method of the present invention more closely approaches the goal of modulating only disease-related T cells while sparing or not affecting other T cells in the subject. The invention therefore achieves a greater specificity of therapy across a wider population.

In a preferred embodiment, a full length T cell antigen receptor V region protein may be used to down-regulate or eliminate a subset of T cells that exhibit a TCR incorporating the same V region. The T cell antigen receptor V region protein can be used in a therapeutic situation to modulate the activity of those T cells bearing the targeted V region or to induce unresponsiveness in the actively responding population of T cells bearing the targeted V region. In this situation the T cell antigen receptor is used as a therapeutic composition in a subject.

Specific T cell antigen receptor V region proteins associated with a given disease are identified using any of a variety of techniques well known in the art. A genetic approach using

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patients known to have myasthenia gravis or multiple sclerosis was described by Oksenberg, J.R., et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:988-992.

The term treatment in the instant invention is meant to include the situations of "prevention," "suppression" or "treatment" of the disease. "Prevention" involves administration of the protective composition prior to the induction of the disease. Thus, for example, in the animal model of experimental autoimmune encephalomyelitis, EAE, successful administration of a protective composition prior to injection of the encephalitogen that induces the disease resulted in "prevention" of the disease.

"Suppression" involves administration of the composition after the inductive event but prior to the clinical appearance of the disease. Again, using the EAE example, successful administration of a protective composition after injection of the encephalitogen, but prior to the appearance of neurological symptoms, resulted in "suppression" of the disease.

"Treatment" involves administration of the protective composition after the appearance of the disease. In the EAE example, successful administration of a protective composition after injection of encephalitogen and after clinical signs had developed resulted in "treatment" of the disease.

For measuring preventative, suppressive or therapeutic benefit of the TCR V region protein of the present invention, certain clinical outcomes are measured. In particular, alteration of proliferative response of lymphocytes *in vitro* can be measured. Assays for measuring the therapeutic benefit of the particular V region protein are known in the art.

Animal model systems which can be used to screen the effectiveness of the proteins in protecting against or treating the disease are available. Systemic lupus erythematosus (SLE) is tested in susceptible mice as disclosed by Knight et al., 1978, *J.Exp. Med.*, 147:1653 and Reinersten et al., 1978, 299:515. Myasthenia gravis (MG) is tested in SJL/J female mice by inducing the disease with soluble AChR protein from another species as described in Lindstrom et al., 1988, *Adv. Immunol.*, 42:233-284. Arthritis is induced in a susceptible strain of mice by injection of Type II collagen as described by Stuart et al., 1984, *Ann. Rev. Immunol.*, 42:233-284. Adjuvant arthritis is induced in susceptible rats by injection of Mycobacterial heat shock protein as described by Van Eden et al., 1988, *Nature*, 331:171-173. Thyroiditis is induced in mice by administration of thyroglobulin as described by Maron et al., 1980, 152:1115-1120. Insulin-dependant diabetes mellitus (IDDM) occurs naturally or can be induced in certain strains of mice such as those described by Kanasawa, et al., (1984). EAE in mice and rats serves as a model for MS in humans. In this model, the demyelinating disease is induced by administration of myelin basic protein (MBP) as described by Paterson, P.Y., Textbook of Immunopathology,

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(Mischer et al., eds.), Grune and Stratton, New York, pp. 179-213 (1986); McFarlin, D.E. et al., 1973, *Science*, 179:478-480: and Satoh, J., et al., 1987, *J.Immunol.*, 138:179-184.

Of course, the identical proteins may not be effective in humans since they may not correspond to an appropriate site of the disease-associated human TCR. It is to be understood that modifications of the protein sequence delineated in a particular animal model may be required in order to treat subjects belonging to other species, including humans.

4. PHARMACEUTICAL COMPOSITIONS CONTAINING THE T CELL ANTIGEN RECEPTOR V REGION

The proteins and compositions of the present invention, or their functional derivatives, are well suited for the preparation of pharmaceutical compositions. The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compositions of the invention. Foremost among such animals are humans, although the invention is not intended to be so limited.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The proteins and pharmaceutical compositions can be administered parenterally by bolus injection or by gradual perfusion over time.

The dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The dose ranges for the administration of the compositions of the present invention are those large enough to produce the desired effect, whereby, for example, an immune response to the proteins as measured by delayed-type hypersensitivity (DTH) or antibody production, is achieved, and the immune-related disease is significantly prevented, suppressed, or treated. The doses should not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Preferred doses for humans range between about 0.001 - 1 mg/kg body weight.

In addition to proteins of the invention which themselves are pharmacologically active, pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferred compositions include the inclusion of an adjuvant, such as alum, or other adjuvants known in the art. (See, for example, Warren et al., 1986, *Ann. Rev. Immunol.*, 4:369-388; Chedid, L., 1986, *Feder. Proc.*, 45:2531-2560.)

To enhance delivery or bioactivity, the proteins can be incorporated into liposomes using methods and compounds known in the art.

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Preparations which can be administered orally in the form of tablets and capsules, preparations which can be administered rectally, such as suppositories, and preparations in the form of solutions for injection or oral introduction, contain about 0.001 to about 99 percent, preferably from about 0.01 to about 95 percent of active compound(s), together with the excipient.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the proteins in water-soluble form, for example, water-soluble salts. In addition, suspensions of the proteins as appropriate oil injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions containing substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The proteins are formulated using conventional pharmaceutically acceptable parenteral vehicles for administration by injection. These vehicles are nontoxic and therapeutic, and a number of formulations are set forth in Remington's Pharmaceutical Sciences (Martin, E.W., ed.) Mack Publishing Co. Easton, PA (1990). Nonlimiting examples of excipients are water,

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saline, Ringer's solution, dextrose solution and Hank's balanced salt solution. Formulations according to the invention may also contain minor amounts of additives such as substances that maintain isotonicity, physiological pH, and stability.

The proteins of the invention are preferably formulated in purified form substantially free of aggregates and other protein materials, preferably at concentrations of about 1.0 ng/ml to 100 mg/ml.

Effective doses of the proteins of this invention for use in preventing, suppressing, or treating an immune-related disease are in the range of about 1 ng to 100 mg/kg body weight. A preferred dose range is between about 10 ng and 10 mg/kg body weight. A more preferred dose range between about 100 ng and 1 mg/kg body weight.

Methods to increase the effectiveness of proteins are known in the art. These techniques may be used in conjunction with the proteins of the instant invention.

The following examples are offered by way of illustration and not limitation.

EXAMPLES

The following general techniques are used throughout the following Examples.

1. GENERAL MATERIALS AND METHODS.

A. Bacteria, plasmids, and general methods

Escherichia coli GM2929, methylase-negative, and *E. coli* LJ24 (*lac* deletion) (Rasmussen et al., 1991, *J. Bacteriology*, 173:6390-6397) were gifts from Dr. Martin Marinus, University of Massachusetts Medical School, Worcester, MA. *E. coli* JM109 (Yanisch-Perron et al., 1985, *Gene*, 33:103-119) were purchased from Promega Corporation, Madison, WI (USA). BL21 (lon^-, $ompT^-$) (Wood, W.B., 1966, *J. Mol. Biol.*, 16:118-133; Studier and Moffatt, 1986, *J. Mol. Biol.*, 189:113-130) were purchased from Novagen, Madison, WI (USA). SG22094 (lon^-, clp^-), SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-) were gifts from Dr. Susan Gottesman, National Institutes of Health, Bethesda, MD (USA). These Gottesman strains have the *lacI* gene deleted. Bacteria were made competent for transformation by the method of Morrison, 1979, *Meth. Enzymology*, 68:326-331) or were purchased (DH5α) from GibcoBRL, Gaithersburg, MD (USA).

pKK233-2 (Amann and Brosius, 1985, *Gene*, 40:183-190) was purchased from Pharmacia, Piscataway, NJ (USA). pBR322 (Bolivar et al., 1977, *Gene*, 2:95-113) was obtained from New England Biolabs, Beverly, MA (USA). pBluescript II KS+ (Alting-Mees et al., 1992, *Meth. Enzymology*, 216:483-495) was obtained from Stratagene, La Jolla, CA (USA). pDC952 containing the *argU* (*dnaY*) gene (Lindsey et al., 1989, *J. Bacteriology*, 171:6197-6205) was a gift from Dr. James R. Walker, University of Texas, Austin, TX. pSE420 (Brosius,

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1992, *Meth. Enzymology*, 216:469-483) and pKK480-3 (Brosius, (1992) *supra*) were gifts from Dr. Jürgen Brosius, Mount Sinai School of Medicine, New York, NY. pUCts (Bukrinsky et al., 1988, *Gene*, 70:415-417) was obtained from Michail I. Bukrinsky via Jürgen Brosius.

Plasmid constructions were performed by standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York(1989)). DNA restriction and modification enzymes were purchased from New England Biolabs, unless otherwise stated, and were used according to the manufacturer's specifications. Synthetic oligonucleotides were purchased from Operon Technologies, Inc., Alameda, CA (USA), unless otherwise indicated.

Oligonucleotides were annealed together by mixing equimolar concentrations of the complementary oligonucleotides in 10 mM Tris pH8.0, 5 mM $MgCl_2$ and placing in 100°C for 5 min and then cooling slowly to 25°C. Polymerase chain reaction (PCR) was performed (Saiki et al., 1988, *Science*, 239:487-491) in a Perkin Elmer Cetus DNA Thermal Cycler, using the GeneAmp reagent kit (The Perkin-Elmer Corporation, Norwalk, CT (USA)). DNA sequencing employed the dideoxynucleotide chain termination method (Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA*, 74:5463-5467) using the Sequenase kit (U.S. Biochemical, Cleveland, OH (USA)). All chemical reagents were of the highest purity available.

B. Growth and induction of cell cultures

For chemically induced cultures, frozen glycerol stocks were used to inoculate 5-25 ml 2XYT medium (16 g Bacto-Tryptone (Difco 0123), 10 g Bacto-Yeast Extract (Difco 0127), 10 g NaCl, pH 7.2-7.4, per liter water) containing 6 µg/ml tetracycline, and allowed to grow overnight at 37°C, 280 rpm. Typically, 1/100 volumes of the overnight culture were used to inoculate each of two 125-ml flasks containing 25 ml 2XYT medium plus 6 µg/ml tetracycline. Cultures were grown at 37°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units, and then induced with 1 mM isopropyl ß-D-thiogalactopyranoside (IPTG), and allowed to grow for a further 2 hr.

For thermal induction, cultures were grown at 30°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units. Thereafter, one culture was maintained at 30°C, and the other was induced by transferring to a water bath at 42°C, and allowed to grow for a further 2 hr. Alternatively, a culture was induced by growing at 30°C until A_{580} was 0.3-0.6 absorbance units, and thereafter was divided into two aliquots; one was maintained at 30°C, and the other was allowed to grow at 42°C.

To assay for results of induction, duplicate samples equivalent to 1 ml of cells at A_{580} = 1.0 were removed (e.g., assuming A_{580} = 3.0, then 333 µl culture was removed). The samples were centrifuged in an Eppendorf microcentrifuge at 14,000 x g, 1 min., the supernatants discarded, and the cell pellets were stored at -20°C.

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C. Stability study

Bacteria were streaked to single colonies three successive times and a single colony from the third plate was used to inoculate 200 ml 2XYT medium containing 6 μg/ml tetracycline. The culture was grown to A_{580} = 0.5-1.0 absorbance units, and 60 ml were removed, mixed with 40 ml glycerol and 1 ml aliquots were distributed into 100 vials to be stored at -80°C. The remainder of the culture was divided into two aliquots; one was incubated uninduced, and the other was induced by IPTG addition or temperature shift. Both cultures were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed prior to and at 2 hr after induction, and assayed for A_{580} absorbance, pH, and protein production by EIA, acrylamide gel electrophoresis, and Western blotting.

Three vials from the frozen bank were tested in parallel for bacterial growth and protein production. Each vial (1 ml) was used to inoculate 200 ml 2XYT medium plus 6 μg/ml tetracycline, and incubated to A_{580} = 0.5-1.0 absorbance units. Thereafter each culture was divided into two aliquots, one to be incubated uninduced, the other to be induced chemically or thermally. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed from both cultures prior to and at 2 hr after induction and assayed as described above.

For the stability study, a 1-ml aliquot was removed from one of the three parallel cultures prior to induction, diluted 1/1000 in 2XYT medium, and 20 μl or 200 μl was inoculated into 200 ml 2XYT medium plus tetracycline, and allowed to grow to A_{580} = 0.5-1.0 absorbance units. Thereafter the culture was divided into two aliquots; one was induced, and both were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed prior to and at 2 hr after induction and assayed as above.

D. Cell fractionation

The cells were pelleted by centrifugation at 7000 x *g*, 7 min, and the supernatant (culture medium) was recentrifuged at 14,000 x *g*, 5 min prior to electrophoresis. The cell pellet was resuspended in 20% (w/v) sucrose, 0.3 M Tris-HCl pH 8.0, 1 mM EDTA using 1/5 of initial culture volume, incubated at 25°C for 15 min, and centrifuged at 6000 x g, 7 min. The supernatant was discarded, and protein located in the periplasm was released by osmotic shock (Nossal and Heppel, 1966, *J. Biol. Chem.*, 241:3055-3062) by resuspending in 1/5 original volume cold water, and incubating at 0°C, 10 min. Following centrifugation at 9000 x *g*, 10 min, the supernatant was saved (periplasmic fraction); the cell pellet was resuspended in 1X TST (25 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, 10 ml/liter Tween 20), sonicated, centrifuged at 12,000 x *g*, 15 min, and the supernatant was saved (soluble cytosolic fraction).

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E. Polyacrylamide gel electrophoresis

The frozen *E. coli* cell pellet was resuspended in 70 µl water by vigorous vortexing or by sonication at 0°C (Sonic Dismembrator, Model 301, ARTEK Systems Corporation) three times each for 5 seconds, at power output 0.4. The disrupted cells were mixed with 70 µl 2X loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% ß-mercaptoethanol, 0.002% Bromophenol Blue) (Laemmli, 1970, *Nature*, 227:680-685.) Samples were incubated at 100°C, 5 min., centrifuged at 14,000 x *g*, 30 seconds, and 10-15 µl aliquots were loaded onto pre-cast 16% or 10-20% acrylamide Tricine gels (Novex, San Diego, CA (USA)) and run in reservoir buffer obtained from the same manufacturer. Electrophoresis was at 125 V constant voltage, or 45 mA constant current, per two gels, for about 2 hr, until the bromophenol blue tracking dye was off the gels. Markers were prestained low molecular weight protein standards (# SE130024, Integrated Separation Systems, Natick, MA (USA)) used at 1 µl per lane. Gels were stained overnight in 10% acetic acid, 25% methanol, 0.025% Coomassie Brilliant Blue R.

F. Western blot analysis

Following separation by SDS-PAGE, proteins were immunodetected (Towbin et al., 1979, *Proc. Natl. Acad. Sci. USA*, 76:4350-4354) by electrotransfer to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA (USA)) using a Semi-Dry electroblotter (Integrated Separation Systems). Proteins were transferred at 10 mA per cm^2 membrane for 1-3 hr, and the membranes were blocked overnight in blocking buffer (1X PBS, 2% dried milk, 0.1% Tween-20).

The membranes were washed three times, 5 min each in 1X PBS, 0.1% Tween-20, followed by washing three times, 5 min each in 1X PBS, and then they were submerged in an horseradish peroxidase (HRP)-conjugated monoclonal anti-Vβ5.3 antibody (13A2-HRP, 0.25 mg/ml) diluted 1:3000 in 1 X PBS, 0.1% Tween-20. Following gentle agitation for 2 hr, the membranes were washed as described above, and the proteins were visualized with the Enhanced Chemiluminescence assay (Amersham, Arlington Heights, IL (USA)) used according to the manufacturer's instructions. Film exposure was between 5 seconds and 1 minute.

G. Enzyme immunoassay

The frozen cell pellets were resuspended by vigorous vortexing in 20 µl 8 M urea, 50 mM Tris-HCl, pH 8.5, (Marston and Hartley, 1990, *Meth. Enzymology*, 182:264-276) and Vβ5.3 was quantified by enzyme immunoassay as follows:

Nunc-Immuno Maxisorp plates (VWR #62409-002) were coated with 100 µl/well 4C2 IgG at 4 µg/ml in PBS, and stored at 4°C overnight. The coating solution was discarded, and blocking buffer (1% casein hydrolysate in PBS, 0.05% Tween-20) was added at 200 µl/well. The plates were incubated at room temperature for 2 hr, 150 rpm, and then washed three times

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with wash buffer (PBS, 0.05% Tween-20). Samples (diluted 1/2,000 ~~to 1/32,000) and Vβ5.3~~ standards in blocking buffer were added at 100 µl/well, and incubated at room temperature for 90 min, 150 rpm. The plates were washed three times with wash buffer, and an HRP-conjugated anti-Vβ5.3 monoclonal antibody (13A2-HRP) diluted 1/500 in blocking buffer plus 5% fetal bovine serum was added at 100 µl/well (0.5 µg/ml). The plates were incubated at room temperature for 90 min, 150 rpm, washed four times, and 100 µl/well O-Phenylenediamine (OPD) substrate was added. After 30 min, the reaction was terminated by adding 50 µl 2N H_2SO_4 per well. The absorbance was measured at 490 nm minus the background absorbance at 650 nm. Blank wells did not contain sample.

2. CONSTRUCTION OF PLASMIDS

EXAMPLE 1

Construction of pK2D

The starting point for the expression of the T cell antigen receptor V region proteins of the invention was the construction of a plasmid that contained the proper regulatory elements for expression of the T cell antigen receptor V region protein. These elements include the promoter, two transcription terminators, an origin of replication for stable replication in bacteria. Additionally because of regulatory concerns associated with the manufacture of a protein for which one possible indication is the *in vivo* use in a therapeutic setting, a gene coding for resistance to tetracycline was chosen to facilitate selection of plasmid bearing cells. Finally, the starting expression vector required a multiple cloning site, strategically engineered to facilitate the cloning of the preferred coding regions of the invention into the expression vector. The following example describes the unique starting plasmid of the invention.

The starting point for the construction of preferred expression vector was pKK233-2 (4.6 kb), described *infra*. The *Nco*I restriction site was converted to a *Spe*I site as follows: pKK233-2 was digested with *Nco*I, and the resulting 5' protruding ends were removed by incubation with mung bean nuclease at 25°C for 30 min. The plasmid was extracted twice with phenol-chloroform, precipitated with ethanol in the presence of 2 M ammonium acetate, and resuspended in water. Following dephosphorylation with bacterial alkaline phosphatase (GibcoBRL) the vector was ligated to a synthetic *Spe*I linker (#1085, New England Biolabs), and the ligation mix was transformed into DH5α competent cells to yield vector pKK233-2A.

Next, the tetracycline gene was restored as follows: pKK233-2A (4.6 kb) was digested with *Eco*RI and *Sal*I yielding two fragments, 279 bp and 4321 bp each. The two fragments were separated by agarose gel electrophoresis, and the large fragment was purified from agarose using the Geneclean Kit (BIO 101, La Jolla, CA (USA)). A 651-bp fragment containing the amino-terminal coding region of the tetracycline gene was excised with *Eco*RI and *Sal*I from

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pBR322, and ligated to the 4321-bp fragment from pKK233-2A. The resulting plasmid pK2B (4.97 kb) conferred tetracycline resistance on *E. coli* DH5α cells, and was further modified to delete a portion of the ß-lactamase gene and convert the *Hind*III site into a *Sac*II site, as follows:

A DNA segment spanning the unique *Spe*I site to the 3' terminus of the transcription terminators was PCR-synthesized using pK2B as template. The 5' "sense" primer contained restriction enzyme recognition sequences for *Spe*I, *Pst*I and *Sac*II in the 5' to 3' direction, underlined:

5' - TATAATGACTAGTCGCTGCAGCCAACCGCGGCTG -3' [SEQ ID NO 1]

The 3' "antisense" primer contained a *Sca*I site, underlined:

5' - GTCCTAGAGTACTGAGCGGATAC - 3' [SEQ ID NO 2]

1 ng of pK2B DNA template was mixed in a reaction volume containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 µg/ml gelatin, 200 µM each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), 1 µM each of the two primers, and 2.5 units AmpliTaq DNA polymerase. The final reaction volume was 100 µl, overlaid with 100 µl mineral oil. The DNA was denatured at 95°C for 2 min, and amplification was achieved with 30 cycles of 1 min at 95°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for primer extension. The amplified DNA was analyzed on 1% agarose gel run in 40 mM Tris-HCl pH 7.8, 5 mM NaAcetate, 1 mM EDTA.

The resulting 485-bp PCR-synthesized fragment was digested with *Spe*I and *Sca*I, and ligated to a 4129-bp fragment that was obtained by digestion of pK2B with *Spe*I and *Sca*I resulting in a plasmid called pK2C.

Plasmid pK2C (4.6 kb) was further modified to contain a multiple cloning site. First, pK2C was digested with *Spe*I and *Sac*II, and ligated to a double-stranded synthetic oligonucleotide containing 15 restriction sites. This multiple cloning site (MCS) was formed by annealing equimolar concentrations of two complementary synthetic oligonucleotides, 91 and 97 bp long, respectively, having the following sequences:

5' - GGCTCGAGCCTAGGCTGCAGCCCGGGGCGCGCGCGGCCGCAGGCC
TTTAATTAAGAGCTCCGGACCGCACAATGTGGGCGCGCCCTTAAGA - 3'
[SEQ ID NO 3]

5' - CTAGTCTTAAGGGCGCGCCCACATTGTGCGGTCCGGAGCTCTTAA
TTAAAGGCCTGCGGCCGCGCGCGCCCCGGGCTGCAGCCTAGGCTCGAG
CCGC - 3' [SEQ ID NO 4]

The MCS codes for the following restriction enzymes sites: *Spe*I, *Afl*II, *Asc*I, *Dra*III, *Rsr*II, *Sac*I, *Pac*I, *Stu*I, *Not*I, *BssH*II, *Xma*I, *Pst*I, *Avr*II, *Xho*I, and *Sac*II. The ligation mix was

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transformed into methylase-negative *E. coli* GM2929. The resulting plasmid pK2D (4.67 kb) was partially sequenced to ensure the integrity of the *in vitro* synthesized regions.

Results

The starting plasmid was pKK233-2 which contains the following elements: The *trc* promoter (Brosius et al., (1985) *supra*) which is similar to the *tac* promoter derived by fusing the -10 region of the *lac*UV5 promoter with the -35 region of the tryptophan promoter (de Boer et al., (1983) *supra*; Amann et al., (1983) *supra*). The plasmid also contains a ribosome binding site followed by a cloning site of three unique restriction enzymes (*Nco*I, *Pst*I and *Hind*III), followed by the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118). In addition, pKK233-2 contains the ß-lactamase gene for ampicillin resistance, the pBR322 origin of replication, and a small non-functional carboxyl terminal fragment from the tetracycline resistance gene.

pKK233-2 was modified in order to destroy the *Nco*I site, restore the tetracycline resistance gene, delete a segment of the ß-lactamase coding region (thus inactivating the gene), and insert a multiple cloning site consisting of 15 restriction sites to produce pK2D.

EXAMPLE 2

Plasmid pK2D was further modified to incorporate either the pelB signal sequence from *Erwinia carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383) or the heat stable enterotoxin II (STII) signal sequence of *Escherichia coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732.)

Construction of pK-pelB.

Plasmid pK2D (4.67 kb) was engineered to include a ribosome binding site (Shine and Dalgarno, 1974, *Proc. Natl. Acad. Sci. USA*, 71:1342-1346; Shine and Dalgarno, 1975, *Nature*, 254:34-38; Steitz and Jakes, 1975, *Proc. Natl. Acad. Sci. USA*, 72:4734-4738) and the pelB signal sequence from *E. carotovora* (Lei et al., (1987) *supra*) to create plasmid pK-pelB (4.74 kb), as follows:

pK2D was digested with *Spe*I and *Sac*I located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 103 bp and 95 bp long, respectively, having the sequence shown below. A silent mutation (lower case letters) was introduced into the pelB sequence, in order to create an *Nco*I restriction site, underlined, facilitating the subsequent subcloning of the Vβ5.3 gene sequence.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAATACC
TATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCA
GCcATGGCCGAGCT - 3' [SEQ ID NO 5]

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5' - CGGCCATgGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCT
GCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGAAATAGA
ATTTA - 3' [SEQ ID NO 6]

Construction of pK-STII.

Plasmid pK2D was engineered to include a ribosome binding site and the heat-stable enterotoxin (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., (1991) *supra*), as follows:

pK2D was digested with *Spe*I and *Dra*III located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 105 bp and 98 bp long, respectively, having the sequence shown below. Two silent mutations (lower case letters) were introduced into the STII sequence, in order to create *Mlu*I and *Dra*III restriction sites , underlined, facilitating the subsequent subcloning of Vβ5.3.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAAAAGAA
TATAGCATTCCTACTAGCTTCAATGTTCGTCTTCTCTATTGCAACTA
ACGCgTACGCaCATT - 3' [SEQ ID NO 7]

5' - GtGCGTAcGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGCT
AGTAGGAATGCTATATTCTTTTTCATTATGACTGTCTCCTTGAAATA
GAATTTA - 3' [SEQ ID NO 8]

EXAMPLE 3

The plasmids containing the signal sequences from Example 2 *supra* were further modified to include two regulatory elements.

Construction of pKT-pelB.

Plasmid pK-pelB was further modified to include two regulatory elements upstream of the pelB leader, to create pKT-pelB (4.8 kb). These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron, ATG TAT CGA TTA AAT AAG GAG GAA TAA (SEQ ID NO.28), coding for 8 amino acid residues. Insertion of the two regulatory elements was achieved by digestion of pK2D with *Spe*I and *Sac*I, followed by ligation of the vector to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the pelB leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 71-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp,

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respectively) and Pair B (oligos J5B + J6B, 71 bp and 76 bp, respectively) having the following sequence:

J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA
AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA
ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5B

5' - ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTC
GCTGCCCAACCAGCCATGGCCGAGCT - 3' [SEQ ID NO 11]

J6B

5' - CGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCTG
CCGTAGGCAATAGGTATTTCATTATTTATTC - 3' [SEQ ID NO 12]

Construction of pKT-STII.

Plasmid pK-STII was further modified to include the same two regulatory elements as in pKT-pelB, thus creating pKT-STII (4.8 kb). pK2D was digested with *Spe*I and *Dra*III, and the vector was ligated to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the STII leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 73-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp, respectively) and Pair B (oligos J5C + J6C, 73 bp and 79 bp, respectively) having the following sequence:

J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA
AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA
ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5C

5' - ATGAAAAAGAATATAGCATTCCTACTAGCTTCAATGTTCGTCTT
CTCTATTGCAACTAACGCGTACGCACATT - 3' [SEQ ID NO 13]

J6C

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5' - GTGCGTACGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGC
TAGTAGGAATGCTATATTCTTTTTCATTATTTATTC - 3' [SEQ ID NO 14]

EXAMPLE 4

Construction of pK-pelB-Vß5.3.

The next step in the expression of the T cell antigen receptor Vβ5.3 protein was the incorporation of the Vβ5.3 sequence into the plasmids containing the signal sequences as described in Example 2 *supra.*

The complete nucleotide sequence of Vβ5.3 was generated as follows: the DNA sequence of Vβ5.3 clone 12A1 was originally published by Leiden et al. (1986, *Proc. Natl. Acad. Sci. USA*, 83:4456-4460) and later partially corrected (Leiden et al., 1986, *Molec. Cell Biol.*, 6:3207-3214). This clone 12A1 was missing nucleotides coding for the N-terminal four amino acid residues; and in addition, the deduced N-terminal amino acid residues of clone 12A1 were incorrect. The N-terminal amino acid sequence of Vβ5.3 (HPB-ALL) was published by Jones et al., 1985, *Science*, 227:311-314) without specifying the N-terminal first amino acid residue, which was later determined to be Glycine (Jeff Leiden, unpublished). Using the above sequence information, the complete Vβ5.3 was reconstructed by recombinant DNA techniques. The resulting plasmid (pBB-Vβ5.3) was the source of the Vβ5.3 sequence for the following examples. The full length nucleotide sequence coding for Vβ5.3 and the predicted amino acid sequence have been published more recently by Plaza et al. (1991, *J. Immunol.*, 147:4360-4365). (See, also, Figure 1; SEQ ID NOS. 25, 26 and 27 herein.)

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TAATTAGCCATGGCCGGCGTAACCCAATCTCCG - 3' [SEQ ID NO 15]

The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 16]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *EcoR*V site of pBluescript II KS+ to yield plasmid pBL-pelB- Vβ5.3. The Vβ5.3 insert was excised by digestion with *Nco*I and *Pst*I, purified from agarose, and ligated into the *Nco*I and *Pst*I sites of pK-pelB, yielding plasmid pK-pelB- Vβ5.3 (5 kb).

Construction of pK-STII-Vß5.3.

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Mlu*I restriction enzyme site, underlined:

5' - GAAATTAACGCGTACGCAGGCGTAACCCAATCTC - 3' [SEQ ID NO 17]

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The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 18]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *EcoR*V site of pBluescript II KS+ to yield plasmid pBL-STII- Vβ5.3. The Vβ5.3 insert was excised by digestion with *Mlu*I and *Pst*I, purified from agarose, and ligated into the *Mlu*I and *Pst*I sites of pK-STII, yielding plasmid pK-STII- Vβ5.3 (5 kb).

Results

Vβ5.3 fused to the pelB or the STII leaders was expressed in *E. coli* JM109 (TABLE 1). Induction was for 2 hr. and Vβ5.3 yields were measured by EIA.

TABLE 1

EXPRESSION AND COMPARTMENTATION OF Vβ5.3

	pK-pelB-Vß5.3		pK-STII-Vß5.3	
Compartment	0 mM IPTG	1 mM IPTG	0 mM IPTG	1 mM IPTG
Supernatant	0 µg/ml	1.3 µg/ml	0.5 µg/ml	2.2 µg/ml
Periplasm	0.9	4.6	N.D.	5.5
Cytosol	9.9	Off scale [1:40]	2.0	Off scale [1:40]

Both leaders were functional, although most of the expressed protein was localized intracellularly.

EXAMPLE 5

This Example details the plasmids containing the leader sequence and the two regulatory elements (g10 and mini-cistron) as well as the Vβ5.3 sequence.

Construction of pKT-pelB-Vß5.3

Plasmid pKT-pelB-Vβ5.3 was constructed by excision of the Vβ5.3 insert from pK-pelB-Vβ5.3 using *Nco*I and *Pst*I, and ligation into the *Nco*I and *Pst*I sites of pKT-pelB.

Construction of pKT-STII-Vß5.3

Plasmid pKT-STII-Vβ5.3 was similarly constructed by excision of the Vβ5.3 insert from pK-STII-Vβ5.3 using *Mlu*I and *Pst*I, and ligation into the *Mlu*I and *Pst*I sites of pKT-STII.

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All subsequent plasmid constructs utilized the STII signal sequence. Therefore, where the signal sequence is not specified, it is understood that the STII sequence was utilized.

Results

pKT-Vß5.3

Plasmid pK-STII-Vβ5.3 (Example 4) was modified to include two regulatory elements upstream of the STII leader, to create pKT-Vβ5.3. These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron coding for 8 amino acid residues. Both elements have been demonstrated to enhance translational efficiency of several genes (Schoner et al., 1984, *Proc. Natl. Acad. Sci. USA*, 81:5403-5407, Schoner et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:8506-8510; Olins et al., 1988, *Gene*, 73:227-235; Olins and Rangwala, 1989, *J. Biol. Chem.*, 264:16973-16976).

pKT-Vβ5.3 provided up to 8-fold higher protein yields than pK-STII-Vβ5.3 (Example 4) in *E. coli* JM109 under conditions of IPTG induction. The EIA values reported here are approximations, due to the lack of a pure Vβ5.3 standard at this time. pKT-Vβ5.3 was shown to be stable in *E. coli* JM109.

EXAMPLE 6

Construction of pKTB-Vß5.3.

A further modification to pKT-Vβ5.3 (Example 5) for secreted expression involved elimination of the IPTG requirement for induction. The existence of a *lacI*ts gene coding for temperature-sensitive *lac* repressor (Bukrinsky et al., 1988, *Gene*, 70:415-417) makes it possible to control gene expression from the *trc* promoter by elevating the temperature from 30°C to 42°C. This would obviate the need to use IPTG or bacterial strains harboring the *lacI* or *lacI*^{q} genes.

The *lacI*ts gene was excised from pUCts by *EcoR*I digestion. The 1.7 kb fragment containing the entire *lacI*ts-coding region and its associated regulatory elements, flanked by vector sequence, was blunt-ended with Klenow, and ligated into the dephosphorylated ScaI site, located downstream of the rrnB operon in pKT-Vβ5.3, yielding plasmid pKTB-Vβ5.3 (6.8 kb). The orientation of the *lacI*ts gene was determined by restriction mapping.

Results

The *lacI*ts gene was inserted in either orientation downstream of the *rrnB* transcription terminators in pKT-Vβ5.3 to create plasmid pKTB-Vβ5.3 (Fig. 2). This was expressed in *E. coli* LJ24 (*lacI* deletion) and the protein was analyzed by Coomassie Blue staining on acrylamide gels (Laemmli, (1970) *supra*) and by Western blot (Towbin et al.,

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(1979) *supra*). One orientation of the *lacI*ts gene (transcription direction toward the Amp^r region) clearly resulted in higher Vβ5.3 yield than the reverse orientation.

The putative Vβ5.3 band obtained from extracts of total cellular pellets was electrotransferred to Immobilon-P membranes, and visualized by Coomassie Blue staining. The band was excised and subjected to NH_2-terminal amino acid sequencing (Matsudaira, 1987, *J. Biol. Chem.*, 262:10035-10038) to yield a total of 10 amino acid residues. These matched precisely the sequence of the N-terminal 10 amino acid residues of the processed Vβ5.3, i.e. no leader sequence was detected.

This plasmid was also expressed by thermal induction in three different bacterial strains, each of which contains increasing levels of endogenous *lac* repressor, i.e., LJ24<DH5α<JM109. Supernatants from induced cultures were assayed for Vβ5.3 expression by EIA (Table2)

TABLE 2

pKTB-Vß5.3 EXPRESSION IN DIFFERENT BACTERIAL STRAINS

Cells were induced for 2 hr., and Vβ5.3 yields (μg/ml) were measured in culture supernatants.

Plasmid	Regulation	*E. coli* Host	Induction	Uninduced	Induced
pKTB-Vβ5.3	*lacI*ts	JM109 (lacI^q)	42°C	N.D.	0.5
pKTB-Vβ5.3	*lacI*ts	DH5α (lacI)	42°C	N.D.	5.0
pKTB-Vβ5.3	*lacI*ts	LJ24 (Δ*lacI*)	42°C	N.D.	5.2
pKT-Vβ5.3	*lacI*^{q}	JM109	2mM IPTG	0	0.9
pKT	*lacI*^{q}	JM109	42°C	N.D.	0

The data agree with Western blot observations and predicted results, i.e., the highest expression level was achieved in LJ24 (Δ*lacI*) and the lowest in JM109 (*lacI*^{q}). The comparatively low amounts of Vβ5.3 measured in the culture supernatants follow from the fact that *E. coli* does not ordinarily secrete proteins into the medium. As shown in Table 1, the bulk of the expressed Vβ5.3 is retained in the periplasm.

DNA sequencing of the *lacI*ts gene.

The entire coding sequence of the temperature-inducible *lacI*ts gene plus the upstream 80 nucleotides including the promoter were sequenced and compared to the published sequence of the IPTG-inducible *lacI* gene (Farabaugh, 1978, *Nature*, 274:765-769). The promoter region was identical to that of the *lacI*, and not *lacI*^{q}, gene (Calos M. P., 1978, *Nature*, 274:762-765). The *lacI*ts gene sequence showed an

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open reading frame of 1080 nucleotides encoding 360 amino acids, and differed from the *lacI* gene at nucleotide #559 (with reference to first nucleotide of the start codon), which changed from G to A, causing amino acid residue #187 to change from Glycine (GGC) to Serine (AGC). This change in a single amino acid residue imparts thermal sensitivity to the *lac* repressor protein.

In addition, the published sequence of the *lacI* gene (Farabaugh, (1978) *supra*) as well as the sequence appearing in Genebank (locus ECOLAC) contains an error at position #857: nucleotide #857 should be T instead of C, which would make residue #286 code for Leucine (TTA) instead of Serine (TCA). The C-T transition introduces a *Hpa*I site at the 3' end of the *lacI* gene that was shown previously by restriction mapping (Brosius, 1992, *Meth. Enzymol.*, 216:469-483). It should be noted that in Farabaugh, (1978) *supra*, the same codon (TCA) is erroneously reported as encoding Leucine instead of Serine.

EXAMPLE 7

Construction of pKTB.

Plasmid pKTB-Vβ5.3 (6.7 kb) was digested with *Sal*I and *Pst*I, and the 5.42 kb fragment was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pKT-STII (4.8 kb) was digested with *Sal*I and *Pst*I, and the 1.15 kb fragment was similarly purified, and ligated to the 5.42 kb fragment, yielding plasmid pKTB (6.56 kb).

EXAMPLE 8

Construction of pKB-Vß5.3.

This plasmid is identical to pKTB-Vβ5.3 minus the two regulatory elements, i.e., the g10 translational enhancer and the mini-cistron. Plasmid pKTB (6.56 kb) was digested with *Sal*I and *Pst*I, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-Vβ5.3 (4.9 kb) was digested with *Sal*I and *Pst*I, and the 1.3-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB-Vβ5.3 (6.7 kb).

Results

pKB-Vß5.3

As discussed above, the addition of two regulatory elements, i.e., the g10 gene segment and the mini-cistron, appeared to enhance Vβ5.3 yield under conditions of IPTG induction. We wished to remove these two elements from the pKTB-Vβ5.3 vector, and reexamine Vβ5.3 yield under conditions of thermal induction. A comparison of pKTB-Vβ5.3 and pKB-Vβ5.3 by acrylamide electrophoresis and Western blotting showed

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that Vβ5.3 yield was unaffected by the removal of these two elements. EIA measurements of whole cell urea extracts showed that pKB-Vβ5.3 produced more protein than pKTB-Vβ5.3 when expressed in *E. coli* LJ24.

TABLE 3

EXPRESSION OF Vβ5.3 IN DIFFERENT BACTERIAL STRAINS

Plasmid	LJ24	SG22094	SG21163	SG21173
pKTB-Vß5.3 + leader + g10/MC	90	80	20	30
pKB-Vß5.3 + leader - g10/MC	110	50	40	20
pKTBi-Vß5.3 - leader + g10/MC	6	100	100	100
pKBi-Vß5.3 - leader -g10/MC	10	170	130	390

Induction period: 2 hr at 42°C
Culture volume: 25 ml
Leader: STII
g10: T7 gene 10
MC: Mini-cistron
Samples: whole cell urea extracts
Vβ5.3 yields (mg/liter), based on units/ml approximations, as measured by EIA. 1 mg Vβ5.3 is approximately equivalent to 1×10^7 units.

Plasmid pKB-Vβ5.3 was demonstrated to be stable for fermentation.

EXAMPLE 9

Construction of pKB

Plasmid pKTB (6.56 kb) was digested with *Sal*I and *Pst*I, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-STII (4.75 kb) was digested with *Sal*I and *Pst*I, and the 1.1-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB (6.5 kb).

EXAMPLE 10

Construction of pKTBi.

This plasmid is identical to pKTB minus the STII signal sequence. pKTB (6.56 kb) was digested with *Spe*I and *Xma*I, and ligated to a double-stranded synthetic oligonucleotide

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coding for the g10 translational enhancer, a ribosome binding site, and a mini-cistron containing a second ribosome binding site. Restriction sites for *Nco*I, *Dra*III, *Rsr*II and *Xma*I were designed in the 3' end. The two complementary oligonucleotides had the following sequences:

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTA
AAGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAACC
ATGGCACATTGTGCGGTCCGC - 3' [SEQ ID NO 19]

5' - CCGGGCGGACCGCACAATGTGCCATGGTTTATTCCTCCTTATTTA
ATCGATACATTAATATATACCTCTTTAATTTTTAATAATAAAGTTA
ATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 20]

EXAMPLE 11

Construction of pKBi

This plasmid is identical to pKB minus the STII signal sequence. pKTB (6.56 kb) was digested with *Spe*I and *Dra*III, and ligated to a double-stranded synthetic oligonucleotide containing a ribosome binding site, and *Nco*I and *Dra*III sites designed in the 3' end. The two complementary oligonucleotides had the following sequences:

5' - CTAGTAAATTATATTTAAAGGAGGAATAAACCATGGCACATT - 3' [SEQ ID NO 21]

5' - GTGCCATGGTTTATTCCTCCTTTAAATATAATTTA - 3' [SEQ ID NO 22]

EXAMPLE 12

Construction of pKTBi-Vß5.3

This plasmid is identical to pKTB-Vß5.3 minus the STII signal sequence. pKTBi (6.4 kb) was digested with *Xma*I and *Nco*I and ligated to PCR-synthesized Vβ5.3 using pKTB-Vβ5.3 as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TATAGTCCATGGGCGTAACC - 3' [SEQ ID NO 23]

The 3' "antisense" primer contained an *Xma*I site, underlined:

5' - AACTTCCCGGGTTATCATTAGCTGC - 3' [SEQ ID NO 24]

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Results

pKTBi-Vß5.3

In parallel with the above work, the STII leader was removed from the expression plasmid. Since most of the expressed Vβ5.3 is retained intracellularly (also see below), it was expected that scale-up of this construct might yield a heterogeneous population of Vβ5.3 molecules, i.e., plus and minus leader, which would complicate subsequent purification of the processed Vβ5.3. Therefore, the STII leader was removed to create pKTBi-Vβ5.3 for intracellular expression. The expressed Vβ5.3 is expected to contain an N-terminal methionine, although this amino acid might be removed by endogenous methionine aminopeptidases (Ben-Bassat et al., 1987, *J. Bacteriology*, 169:751-757; Baneyx and Georgiou, "Expression of Proteolytically Sensitive Polypeptides in *Escherichia coli*", in Stability of Protein Pharmaceuticals, Part A: Chemical and Physical Pathways of Protein Degradation (Tim J. Ahern and Mark C. Manning, eds.), Plenum Press, New York, pp. 69-108 (1992)), as observed for pKBi-Vβ5.3 (below).

pKTBi-Vβ5.3 was completed, but it failed to express significant levels of protein in *E. coli* LJ24 (see Table 3, *supra*). The entire Vβ5.3 insert was sequenced and determined to be unambiguously correct. Plasmid DNA isolated post-induction and analyzed by restriction digestion appeared to be unaltered. One possible explanation for the apparent failure of the LJ24 strain containing pKTBi-Vβ5.3 to express significant levels of Vβ5.3 protein is that the absence of a leader peptide destabilizes intracellular Vβ5.3 protein, and leads to its rapid proteolysis.

Therefore, pKTBi-Vβ5.3 was transformed into the protease-deficient strains SG22094 (lon^-, clp^-), and BL21 (lon^-, $ompT^-$) which gave decreasing yields in the order of SG22904>BL21. The effect of different induction temperatures on yield was also examined, and for both strains 42°C was optimal. In addition, there was no significant increase in production between 2 and 5 hr.

pKTBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-). The results are summarized in Table 3 (*supra*).

It should be noted that strains LJ24, SG22094, SG21163 and SG21174 do not produce endogenous *lac* repressor because the *lacI* gene is deleted in all of these strains. Thus, the wild type *lac* repressor does not interfere with the thermal induction of the *trc* promoter. The foregoing examples show that Vβ5.3 constucts without a signal peptide can be readily expressed in protease deficient hosts, resulting in production of significant amounts of Vβ5.3 protein.

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EXAMPLE 13

Construction of pKBi-Vß5.3

This plasmid is identical to pKTBi-Vβ5.3 minus the g10 element and the mini-cistron (SEQ ID NO. 28). Plasmid pKBi (6.43 kb) was digested with *Nco*I and *Sal*I, and a 970 bp fragment was purified from agarose. pKTBi-Vβ5.3 (6.75 kb) was digested with *Nco*I and *Sal*I and the 5.68 kb fragment was similarly purified, and ligated to the 970 bp fragment, yielding plasmid pKBi-Vβ5.3 (6.64 kb).

Results

pKBi-Vß5.3

This leaderless Vβ5.3 construct minus the two regulatory elements was successfully expressed in protease-deficient strains; our observations are similar to those for pKTBi-Vβ5.3. pKBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-). The results are summarized in Table 3. pKBi-Vβ5.3 consistently produced more protein than pKTBi-Vβ5.3 in all strains tested (Table 3). N-terminal sequencing of the expressed protein showed that the first 17 amino acid residues matched the expected Vβ5.3 sequence, and the terminal methionine residue was absent. The plasmid was also demonstrated to be stable.

The present invention is not meant to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modification are intended to fall within the scope of the appended claims. Various publications are incorporated by reference in their entirety.

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SEQUENCE LISTING

(1)GENERAL INFORMATION:

(i)APPLICANT:T Cell Sciences, Inc

(ii)TITLE OF INVENTION:T Cell Antigen Receptor V Region Proteins and Methods of Preparation Thereof

(iii)NUMBER OF SEQUENCES:29

(iv)CORRESPONDENCE ADDRESS:
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(C)CITY:Needham
(D)STATE:Massachusetts
(E)COUNTRY:United States of America
(F)ZIP:02194-2725

(v)COMPUTER READABLE FORM:
(A)MEDIUM TYPE:Diskette, 3.50 inch, 1.44Mb storage
(B)COMPUTER:IBM PC Compatible
(C)OPERATING SYSTEM:MS-DOS
(D)SOFTWARE:WordPerfect 6.0

(vi)CURRENT APPLICATION DATA:
(A)APPLICATION NUMBER:
(B)FILING DATE:
(C)CLASSIFICATION:

(vii)PRIOR APPLICATION DATA:
(A)APPLICATION NUMBER:08/181,492
(B)FILING DATE:13-January-1994

(viii)ATTORNEY/AGENT INFORMATION:
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(ix)TELECOMMUNICATION INFORMATION:
(A)TELEPHONE:617-345-9100
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(2)INFORMATION FOR SEQ ID NO: 1:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:34 base pairs
(B)TYPE:nucleic acid
(C)STRANDEDNESS:single
(D)TOPOLOGY:linear

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(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TATAATGACT AGTCGCTGCA GCCAACCGCG GCTG 34

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:23 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTCCTAGAGT ACTGAGCGGA TAC 23

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:91 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

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(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGCTCGAGCC TAGGCTGCAG CCCGGGGCGC GCGCGGCCGC AGGCCTTTAA 50

TTAAGAGCTC CGGACCGCAC AATGTGGGCG CGCCCTTAAG A 91

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTAGTCTTAA GGGCGCGCCC ACATTGTGCG GTCCGGAGCT CTTAATTAAA 50

GGCCTGCGGC CGCGCGCGCC CCGGGCTGCA GCCTAGGCTC GAGCCGC 97

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAATA CCTATTGCCT 50

ACGGCAGCCG CTGGATTGTT ATTACTCGCT GCCCAACCAG CCATGGCCGA 100

GCT 103

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:95 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

GGCAATAGGT ATTTCATTAT GACTGTCTCC TTGAAATAGA ATTTA 95

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:105 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

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(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAAAA GAATATAGCA 50

TTCCTACTAG CTTCAATGTT CGTCTTCTCT ATTGCAACTA ACGCGTACGC 100

ACATT 105

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:98 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATGACTGTC TCCTTGAAAT AGAATTTA 98

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:89 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAATA 89

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTCCTTATTT AATCGATACA TTAATATATA CCTCTTTAAT TTTTAATAAT 50

AAAGTTAATC GATGCCCAAT TCCGGA 76

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGAAATACC TATTGCCTAC GGCAGCCGCT GGATTGTTAT TACTCGCTGC 50

CCAACCAGCC ATGGCCGAGC T 71

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

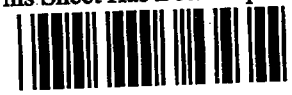
GGCAATAGGT ATTTCATTAT TTATTC 76

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATGAAAAAGA ATATAGCATT CCTACTAGCT TCAATGTTCG TCTTCTCTAT 50

TGCAACTAAC GCGTACGCAC ATT 73

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:79 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATTTATTC 79

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:33 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAATTAGCCA TGGCCGGCGT AACCCAATCT CCG 33

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:34 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:
(ix) FEATURE:

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(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GAAATTAACG CGTACGCAGG CGTAACCCAA TCTC 34

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

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CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAACCA TGGCACATTG 100

TGCGGTCCGC 110

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CCGGGCGGAC CGCACAATGT GCCATGGTTT ATTCCTCCTT ATTTAATCGA 50

TACATTAATA TATACCTCTT TAATTTTTAA TAATAAAGTT AATCGATGCC 100

CAATTCCGGA 110

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:42 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:

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(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTAGTAAATT ATATTTAAAG GAGGAATAAA CCATGGCACA TT 42

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:35 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTGCCATGGT TTATTCCTCC TTTAAATATA ATTTA 35

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:20 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

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TATAGTCCAT GGGCGTAACC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:25 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AACTTCCCGG GTTATCATTA GCTGC 25

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA 39

GGA CAG CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG 78

CAC AAG AGT GTG TCC TGG TAC CAA CAG GTC CTG GGT CAG 117

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GGG CCC CAG TTT ATC TTT CAG TAT TAT GAG AAA GAA GAG 156

AGA GGA AGA GGA AAC TTC CCT GAT CGA TTC TCA GCT CGC 195

CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT GTG AAC GCC 234

TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC AGC 273

AGC 276

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CCG CAT TGG GTT AGA GGC TGA GTG GAC TAG TTT TGC TCT 39

CCT GTC GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC 78

GTG TTC TCA CAC AGG ACC ATG GTT GTC CAG GAC CCA GTC 117

CCC GGG GTC AAA TAG AAA GTC ATA ATA CTC TTT CTT CTC 156

TCT CCT TCT CCT TTG AAG GGA CTA GCT AAG AGT CGA GCG 195

GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA CAC TTG CGG 234

AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG TCG 273

TCG 276

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:92 amino acids

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(B) TYPE:amino acid
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:protein

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln
 1               5                  10                  15
His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val
            20                  25                  30
Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe
            35                  40                  45
Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp
            50                  55                  60
Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn
            65                  70                  75
Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala
            80                  85                  90
Ser Ser
    92
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:27 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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ATG TAT CGA TTA AAT AAG GAG GAA TAA 27

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL:

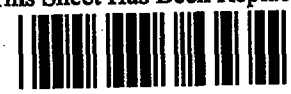
(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Met Tyr Arg Leu Asn Lys Glu Glu
1 5 8

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We claim:

1. A method for producing a recombinant full length T cell antigen receptor V region protein which comprises;

a) transforming a competent bacterial host cell with a recombinant expression vector comprising a region coding for a full length T cell antigen receptor V region protein, and an inducible bacterial promoter;

b) inducing the inducible bacterial promoter in the transformed bacterial host cell of step a, and;

c) recovering the recombinant full length T cell antigen receptor V region protein.

2. The method of claim 1 wherein the inducible bacterial promoter is selected from the group consisting of chemically regulated promoters and thermally regulated promoters.

3. The method of claim 2 wherein the expression vector further comprises a regulatory gene sequence selected from the group consisting of the *lacI* gene or the *lacI*^{q} gene.

4. The method of claim 2 wherein the expression vector includes the *lacI*ts gene.

5. The method of claim 4 wherein the *lacI*ts gene is in the same orientation as the coding region for T cell antigen receptor V region protein.

6. The method of claim 1 wherein the bacterial promotor is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

7. The method of claim 6 wherein the recombinant expression vector further comprises a leader sequence.

8. The method of claim 7 wherein the leader sequence is selected from the group consisting of heat stable enterotoxin STII leader from *Escherichia coli* and a pelB leader from *Erwinia carotovora.*



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9. The method of claim 8 wherein the recombinant expression vector further comprises a leader sequence derived from the STII leader sequence of *E. coli* and the competent bacterial host cell is a cell which does not harbor the *lacI* or *lacI*^{q} gene.

10. The method of claim 9 wherein the bacterial host strain is a protease deficient strain.

11. The method according to claim 10, wherein the protease deficient strain is selected from the group consisting of SG22094, SG21163, and SG21173.

12. The method according to claim 1, wherein the expression vector further comprises the g10 and the mini-cistron sequence (SEQ ID NO. 28) regulatory elements.

13. The method of claim 1 wherein the recombinant expression vector further comprises;

a) a *trc* promotor as the inducible bacterial promoter;

b) a ribosomal binding site;

c) a transcription terminator;

d) a *lacI*ts regulatory gene sequence;

e) a pBR322 origin of replication, and;

f) a tetracycline resistance gene.

14. The method of claim 13 wherein the recombinant expression vector is selected from the group consisting of pKTBi-Vß5.3, and pKBi-Vß5.3.

15. The method of claim 13 wherein the recombinant expression vector further comprises a leader sequence.

16. The method of claim 15 wherein the recombinant expression vector is selected from the group consisting of pK-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, and pKB-Vß5.3.

17. An expression vector comprising a inducible bacterial promoter under the control of a *lacI*ts gene, wherein said *lacI*ts gene controls the expression of a region encoding a full length T cell antigen receptor V region protein.



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18. The expression vector of claim 17 wherein the inducible bacterial promoter is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

19. The expression vector of claim 17 wherein the inducible bacterial promoter is the *trc* promoter.

20. The expression vector of claim 19 which further comprises a leader sequence.

21. An expression vector which comprises in 5' to 3' direction;

a) a *trc* promotor;

b) a ribosomal binding site;

c) a region encoding a full length T cell antigen receptor V region protein;

d) a transcription terminator;

e) a *lacI*ts regulatory gene sequence;

f) a pBR322 origin of replication, and;

g) a tetracycline resistance gene.

22. A bacterial cell transformed with the expression vector according to claim 17.

23. The expression vector of claim 17 wherein said vector is selected from the group consisting of pK-STII-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, pKTBi-Vß5.3, and pKBi-Vß5.3.

24. A full length T cell antigen receptor V region protein produced by the method of claim 13.

25. A pharmaceutical composition which comprises a full length T cell antigen receptor V region protein.

26. The pharmaceutical composition of claim 25 wherein the T cell antigen receptor V region protein is the Vß5.3 protein and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.



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27. A method for treating an immune related disorder comprising administering to a subject in need of such treatment a pharmaceutical composition comprising a full length T cell antigen receptor V region protein.

28. The method according to claim 27 wherein the immune related disorder is multiple sclerosis.

29. The method of claim 28 wherein the full length T cell antigen receptor V region protein is the Vß5.3 and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.

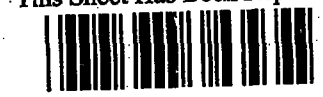
30. A substantially pure T cell antigen receptor Vβ5.3 protein having the formula: amino acids 1-92 of amino acid Sequence ID No. 27.

31. The use of a substantially pure T cell antigen receptor V region protein in the manufacture of a medicament for treatment of an immune-related disease.

32. A transformed bacterial cell which produces at least 300 milligrams of full length recombinant T cell antigen receptor Vβ protein per liter of cell culture.

33. A method of diagnosing an immune disorder comprising using a recombinant full length T cell antigen receptor V region protein to detect in a biological sample from a patient the presence of a particular subpopulation of T cells associated with an immune disorder or the presence of autoantibodies recognizing the particular V region protein.

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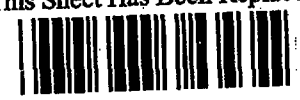
T CELL ANTIGEN RECEPTOR V REGION PROTEINS AND METHODS OF PREPARATION THEREOF

ABSTRACT

The present invention relates to the production of full length T cell antigen receptor V region proteins. The invention provides for methods of expressing the proteins as well as expression vectors for enhanced production of full length T cell antigen receptor V region proteins. The invention further provides for the Vβ5.3 region protein. The proteins of the invention have uses in the diagnosis and therapy of immune-related disorders.

FIGURE 2.

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Figure 1

```
  1  GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA GGA CAG
     CCG CAT TGG GTT AGA GGC TGA CTG GAC TAG TTT TGC TCT CCT GTC
  1  Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln

 46   CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG CAC AAG AGT GTG
      GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC GTG TTC TCA CAC
 16   His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val

 91   TCC TGG TAC CAA CAG GTC CTG GGT CAG GGG CCC CAG TTT ATC TTT
      AGG ACC ATG GTT GTC CAG GAC CCA GTC CCC GGG GTC AAA TAG AAA
 31   Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe

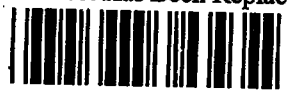
136   CAG TAT TAT GAG AAA GAA GAG AGA GGA AGA GGA AAC TTC CCT GAT
      GTC ATA ATA CTC TTT CTT CTC TCT CCT TCT CCT TTG AAG GGA CTA
 46   Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp

181   CGA TTC TCA GCT CGC CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT
      GCT AAG AGT CGA GCG GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA
 61   Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn

226   GTG AAC GCC TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC
      CAC TTG CGG AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG
 76   Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala

271   AGC AGC     [SEQ ID NO. 25]
      TCG TCG     [SEQ ID NO. 26]
 91   Ser Ser     [SEQ ID NO. 27]
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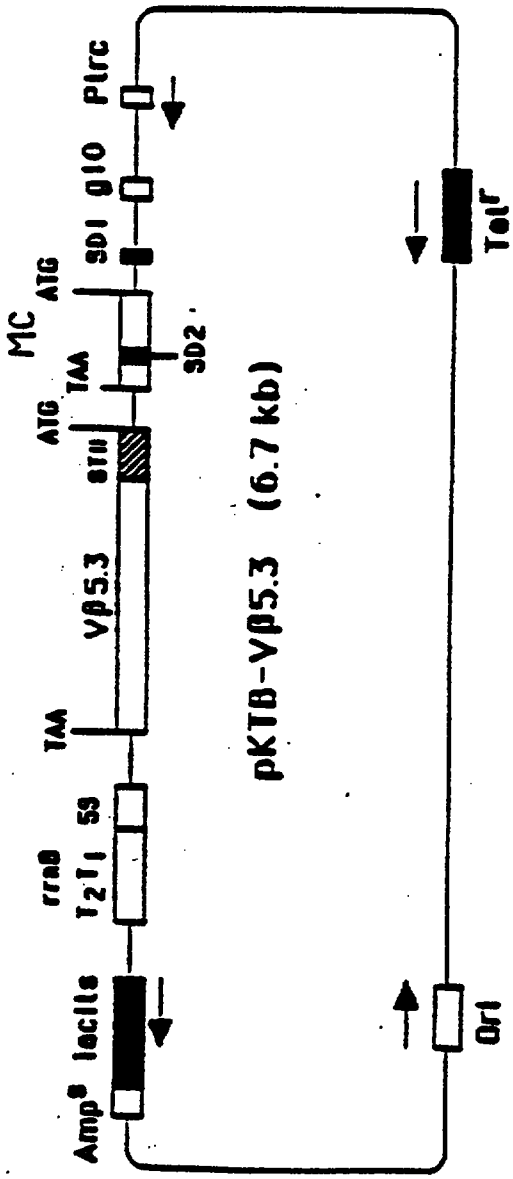
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Figure 2



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T CELL ANTIGEN RECEPTOR V REGION PROTEINS AND METHODS OF PREPARATION THEREOF

INTRODUCTION

The present invention is generally in the fields of immunology and medicine and relates to full length recombinant T cell receptor (TCR) V region proteins, expression vectors for producing commercially useful amounts of recombinant T cell antigen receptor V region proteins, and methods of using full length recombinant T cell receptor V region proteins to diagnose and treat diseases or disorders of the immune system.

BACKGROUND OF THE INVENTION

1. THE T CELL ANTIGEN RECEPTOR

The immune response to foreign antigens is divided into humoral immunity, mediated by B cells, and cellular immunity, mediated by T cells. The receptor molecules used by B cells to recognize antigen are immunoglobulins which recognize conformational epitopes on globular protein. The antigen receptors on T cells are related to immunoglobulins, but bind processed antigen molecules in association with self major histocompatibility complex (MHC) molecules (Dembic et al., 1986, *Immunol. Today*, 7:308).

TCR genes, like immunoglobulin genes, consist of coding regions (or gene segments) which rearrange during T cell ontogeny (Chien et al., 1984, *Nature*, 312:31-35; Hedrick et al., 1984, *Nature*, 308:149-153; Yanagi et al., 1984, *Nature*, 308:145-149). The human T cell receptor ß chain locus has been extensively studied since the cloning of the first cDNA encoding the ß-chain (Yanagi, Y., et al. 1984, *Nature*, 308:145-149; Hedrick et al., 1184, *Nature*, 308:149-152; Siu et al., 1984, *Cell*, 37:393-401). This locus is a gene complex comprising regions encoding known regions or domains of the β chain: the variable (V), diversity (D), and joining (J) domains. These coding regions participate in somatic cell rearrangement with a region encoding the constant (C) domain (Chien et al., 1984, *Nature*, 309:322-326). *In situ* studies identified the location of the TCRß locus on chromosome 7 at 7q35 (Isobe et al., 1985, *Science*, 228:580). By current estimates, this complex spans more than 600 kb and contains 70 to 80 variable region segments (Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Tillinghast et al., 1986, *Science*, 233:879-883; Kimura et al., 1987, *Eur. J. Immunol.*, 17:375-383; Lai et al., 1988, *Nature*, 331:543-546). These V region gene segments are adjacent to two tandemly organized regions, each of which includes a D and a C gene segment followed by a cluster of six or seven J region gene segments (Tunnacliffe et al., 1985, *Nucleic Acids Res.*, 13:6651-6661); Toyonaga et al., 1985, *Proc. Natl. Acad. Sci. USA*, 82:8624-8628). Following successful DNA rearrangement of the V, D, and J coding regions (gene segments), the translated

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ß chain polypeptide pairs with a TCR α chain and can be expressed on the surface of the T cell (reviewed by Kronenberg et al., 1986, *Ann. Rev. Immunol.*, 4:529-591).

The TCR α and δ locus are next to one another on human chromosome 14. TCR δ coding regions are located entirely within the α gene locus (Satyanarayana et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:8166-8170; Chien et al., 1987, *Nature*, 330:722-727; Elliott et al., 1988, *Nature*, 331:627-631). It is estimated that there are a minimum of 45-50 Vα coding regions (Becker et al., 1985, *Nature*, 317:430-434), whereas there are only approximately 10 Vδ coding regions (Chien et al., 1987, *supra*). In peripheral blood, two Vδ coding regions appear to be predominantly expressed, namely, Vδ 1 and Vδ 2.

The γ TCR gene was identified, first in mice (Saito et al., 1984, *Nature*, 309:757-762; Kranz et al., 1985, *Nature*, 313:762-755; Hayday et al., 1985, *Cell*, 40:259-269) and then in humans (Lefranc et al., 1985, *Nature*, 316:464-466; Murre et al., 1985, *Nature*, 316:549-552). The human γ TCR locus appears to consist of between five and ten V, five J and two C coding regions (Dialynas et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:2619).

TCR diversity, and thereby T cell specificity, is derived from several sources (Barth et al., 1985, *Nature*, 316:517-523; Fink et al., 1986, *Nature*, 321:219-225): the multiplicity of germline gene segments (Chien et al., 1984, *Nature*, 309:322-326; Malissen et al., 1984, *Cell*, 37:1101-1110; Gascoigne et al., 1984, *Nature*, 310:387-391; Kavaler et al., 1984, *Nature*, 310:421-423; Siu et al., 1984, *Nature*, 311:344-349; Patten et al., 1984, *Nature*, 312:40-46); combinatorial diversity through the assembly of different V, D, and J coding regions (Siu et al., 1984, *Cell*, 37:393-401; Goverman et al., 1985, *Cell*, 40:859-867); and junctional flexibility, N-region diversity and the use of either multiple D regions or any of the three translational reading frames for Dß segments. TCR diversity does not appear to arise from the somatic hypermutation mechanism observed for immunoglobulins (Barth et al., *supra*). As a result of these mechanisms, TCRs are generated which differ in their amino-terminal, or N-terminal, domains (i.e., are constructed from combinations of V, D, and J coding regions) but are similar elsewhere, including their carboxyl-terminal, or C-terminal domains (which constitute constant regions of TCRs). Accordingly, an extremely large repertoire of TCR molecules is generated.

Study of the structure and diversity of the human TCR ß-chain variable coding regions has led to the grouping of these coding regions into distinct V region subfamilies (Tillinghast et al., 1986, *Science*, 233:879-883; Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602; Borst et al., 1987, *J. Immunol.*, 139:1952-1959). Each V region subfamily can be characterized by a consensus sequence and contains similar coding regions exhibiting greater than about 75% similarity in the DNA sequence. For instance, sixteen mouse Vß sequences have been categorized into fourteen different subfamilies by a proposed arbitrary, but simple,

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numerical nomenclature for the Vβ gene segments (see Barth et al., 1985, "The Murine T Cell Receptor Employs a Limited Repertoire of Expressed Vß Gene Segments", *Nature*, 316:517-523). According to this nomenclature, members of the same subfamily share the first digit and differ in second; therefore the Vß8.1, Vß8.2 and Vß8.3 are all members of the Vß8 subfamily. A similar system has been proposed for the human coding regions. In humans, the approximately 60 functional Vß coding regions have been grouped into at least 24 families (Toyonaga and Mak, 1988, *Ann. Rev. Immunol.*, 5:585; Wilson et al., 1988, *Immunol. Rev.*, 101:149; and Robinson, M.A., 1991, *J. Immunol.*, 146:4392).

2. T CELLS IN AUTOIMMUNITY

T cells play a pivotal role in the differentiation and regulation of effector mechanisms within the immune system (Paul et al., 1987, *Science*, 195:1293-1300). The co-recognition of antigen and major histocompatibility molecules by a T cell must be specific and precisely regulated, since improper immune regulation fosters autoimmunity. Several laboratories have studied diseases in which there appears to be improper immune regulation, such as autoimmunity and some forms of immunodeficiency, and have implicated T cells in the pathogenesis of such diseases.

Several undesirable immune responses are characterized by a clonal or oligoclonal expansion of a particular TCR composition as, for example, in conditions of malignancy which have resulted in a T cell leukemia or lymphoma. (See, e.g., Jack et al., 1990, *Am. J. Pathol.*, 136:17; Nitta, et al, 1990, *Science*, 249:672; and, Yoshino et al., 1991, *Int. J. Cancer*, 47:654). In T cell leukemias or lymphomas, the TCR acts as a unique tumor marker because it is stably rearranged and presented on the surface of the cell. A particular TCR composition is also implicated in the recipient of an organ graft whose T lymphocytes have TCRs reactive with the MHC antigens of the donor individual, as for instance the donor of a bone marrow graft.

Several groups have reported selective TCR V region expression in certain autoimmune situations. For instance, Grunwald et al., have noted a preferential expression of the Vα2.3 gene product in CD4+ T cells in the bronchoalveolar lavage when compared to peripheral blood lymphocytes of patients with Sarcoidosis (Grunwald, J. et al., 1992, *Eur J. Immunol.*, 22:129). In Kawasaki disease the preferential expansion of Vß2 and Vß8 T cells was noted at the onset of disease (Abe, J., et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066).

Rheumatoid arthritis (RA) has been extensively studied in this regard. Several investigators have noted preferential expansion of certain of T cell subsets, e.g., DerSimonian, et al., 1993, *J. Exp. Med.*, 177:1623 (preferential expansion of Vα12.1-bearing T cells in CD8+ peripheral blood T lymphocytes); Stamenkovic, et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:1179 (synovial membrane-infiltrating T cells grown in IL-2 were oligoclonal by Southern

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blot analysis); Paliard, et al., 1991, *Science*, 253:325 (hypothesizing that superantigen-activated Vß14 T cells, including autoreactive T cells, expand clonally and migrate to the synovial fluid of RA patients); Howell et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:10921 (noting particularly Vß3, 14, and 17 T cell V region gene usage in IL-2R+ cells from synovial fluid of RA patients); Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8534 (oligoclonal T cell V region gene usage in synovial fluid T cells of a single RA individual); International Patent Publication WO 90/06758 (implicating Vß3, 9, and 10 in RA).

Inflammatory bowel disease has also been extensively studied. Several groups have noted expanded T cell population or preferential TCR V region gene usage, e.g., Posnett et al., 1990, *J. Clin. Invest.*, 85:1770; Spencer et al., 1991, *J. Clin. Pathol.*, 44:915; Trejdosiewicz et al., 1991, *Clin. Exp. Immunol.*, 84:440; Van Kerckhove et al., 1992, *J. Exp. Med.*, 175:57. Still others have reported preferential TCR V gene usage in *Mycobacterium leprae* (van Shooten et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:11244; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188.

Multiple sclerosis (MS) is an autoimmune disease characterized by central nervous system mononuclear cell infiltration and demyelination. Although the pathogenesis of MS is unknown, both genetic and environmental factors have been implicated in the disease process. Major elements of the genetic predisposition include an association of disease with particular class II major histocompatibility complex (MHC) haplotypes (Terasaid et al., 1976, *Science*, 1933:1245-1247; Ho et al., 1982, *Immunogenetics*, 15:509-517: Spielman et al., 1982, *Epidemial. Rev.*, 4:45-65; Francis et al., 1986, *Lancet*, 1:211; Elian et al., 1987, *Disease Markers*, 5:89-99 ; Urban et al., 1988, *Cell*, 54:577-592; Vandenbark, et al., 1989, *Nature*, 341:541-544; Howell et al., 1989, *Science*, 246:668-670).

It has been shown that T cells isolated from the cerebral spinal fluid of patients suffering from MS utilize a limited set of TCR V region genes. The demonstration of *in vivo* activated myelin basic protein (MBP)-specific T cells in MS patients implicates MBP-reactive T cells in the pathogenesis of the disease (Wucherpfennig, K.W., et al., 1990, *Science*, 248:1016-1019). Studies using polymerase chain reaction (PCR) amplification of cDNA with TCR Vß primers indicated that MBP-reactive T cell lines use only a limited number of Vß coding regions (Wucherpfennig, K. W., et al., 1990, *supra* (Vß17, and to a lesser extent Vß12, are frequently used in the recognition of the immunodominant region of the human autoantigen MBP); Oksenberg J. R., et al., 1993, *Nature*, 362:68-70 (rearranged Vß5.2 and/or Vß5.3 genes were detected in the brains of patients with a certain HLA phenotype); Kotzin, B. L., 1991, *Proc. Natl. Acad. Sci. USA*; 88:9161-9165 (a bias for use of the ß chain variable region 5.2/5.3, and to a lesser extent Vß 6.1, seen among MBP-specific clones from patients with MS)). The results of

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some studies also imply that there is a limited TCR Vα gene expression in MS brain lesions (Oksenberg, J. R., et al., 1990, *Nature*, 345:344-346).

3. APPROACHES TO T CELL MEDIATED THERAPY

In the past, efforts to selectively alter the T cell population of an individual have focussed on vaccination with whole live or attenuated T lymphocytes (for a review of this approach see Cohen, I.R.,1986, *Immunol. Rev.*, 94:5-21) or with peptide vaccines based on selecting a specific peptide portion of the TCR V region. These approaches have been based on the current view that potentially pathogenic T cells preferentially utilize a limited set of V coding regions. The approach developed by Cohen uses live or attenuated T cells as a vaccine to treat or prevent, *inter alia*, experimental autoimmune encephalomyelitis (EAE). However, this approach is limited because it depends on the isolation if clones of disease-specific T cells: it is necessary to treat each patient with his own expanded cells, thus requiring "tailor-made" or individualized therapeutics. (See, Zhang, et al., 1993, *Science*, 261:1451).

Others have suggested the therapeutic use of TCR peptides based on TCR V region involvement (Offner, H., 1991, *Science*, 251:430 (treatment with a peptide based on the TCR Vß8 amino acid sequence reduced severity of disease and accelerated recovery in Lewis rats); Hashim, G.A., et al., 1992, *J. Immunol.*, 149:2803-2809; Offner, H. et al., 1992, *J. Immunol.*, 148:1706-1711; Offner, H., et al., 1991, *J. Immunol.*, 146:4165-417; *but see* Sun, D., 1992, *Cell. Immunol.*, 141:200-210). These peptides are generally short peptides, usually between 8-25 amino acids in length, and rarely more than 30 amino acids in length. For example a 21 amino acid peptide present on the TCR chain associated with EAE has been used to prevent EAE in Lewis rats (Vandenbark A.A. et al., 1989, *Nature*, 341:541-544; Vandenbark, A.A., International Patent Publication WO 91/01133; see also, Janeway, C.A., 1989, *Nature*, 341:482-483). Others have reported that even shorter peptides of 8 or 11 amino acids, corresponding to portions of the ß chain VDJ region or the Jα region proved effective as vaccines for either preventing or reducing the severity of EAE in a rat model (Howell, M.D. et al., 1989, *Science*, 246:668-671; Howell, M.D. et al., International Patent Publication WO 92/12996).

Peptides derived from TCR proteins have commonly been chosen as candidates for autoimmune therapeutics because of the ease of peptide synthesis and because no method has been previously available for the production of longer TCR V region proteins in useful quantities and having significant biological activity. More importantly, the major drawback of small synthetic peptides is that their use requires a prediction of the particular sequence that would be effective in modulating an immune response. Predictions must be made as to the antigenic or immunogenic properties of the proposed peptides, as for example their occurrence in the hypervariable or complementarity-determining region (CDR) of a V region. Additionally,

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the size of the proposed peptide derived from a particular TCR must be calculated to maintain the proper epitope structure to prime suppressor T cell activity or to elicit release of antibodies directed against the subpopulation of T cells bearing that particular TCR (to which the peptide corresponds). Such peptide design requires substantial time and experimentation; and in many instances where human therapies are concerned, the necessary experimentation may not be performed, for humane or ethical reasons.

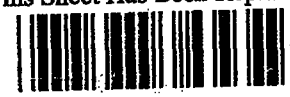
In addition, in order to obtain a specific antigenic response by a regulatory T cell, the peptide must be bound to an individual's MHC molecules. These MHC antigens present on target cells have polymorphic regions wherein specific alleles are associated with particular individuals. For the most part, the individual will have two haplotypes, meaning that there will be two different alleles of a particular MHC antigen from the same locus. Not all peptides will have the capacity to bind to MHC antigens, nor will all MHC antigens bind to any single peptide, since there are multiple MHC antigens for each haplotype. Therefore, the suitability of such a peptide approach to T cell-mediated therapeutics must be determined on the haplotype of each individual, requiring tailoring of peptides to the individual patient.

4. PRODUCTION OF SOLUBLE T CELL ANTIGEN RECEPTOR

Production of large amounts of soluble TCR proteins by recombinant techniques has been problematic for several reasons. A major obstacle to overcome in the production of soluble T cell antigen receptor is the secretion in the absence of the transmembrane region (Traunecker, A. et al., 1989, *Immunol. Today*, 10:29-32). One investigator was able to show secretion of a full length monomeric ß chain of the murine TCR including the V, D, and J regions followed by a truncated form of the Cß region. The methodology took advantage of the homology between the carboxyl-terminal end of the immunoglobulin light chain and the sequence around Cys 241 in the TCR ß chain. The resulting construct utilized the chimeric gene encoding the k-chain leader peptide sequence from MPC-11 and a rearranged VDJ exon. The resulting construct was expressed in mammalian cells and was detectable in the cell supernatant. The method did not suggest, however, a means of obtaining a full length V region protein in the absence of extraneous protein sequences, since secretion was dependant on the presence of the truncated Cß exon (Gasgoigne, N., 1990, *J. Biol. Chem.*, 265(16):9296-9301).

Another group attempted to achieve the expression of a soluble form of the TCR ß chain in biochemically significant quantities by the expression of a recombinant TCR heterodimer anchored by a phosphatidyl inositol glycan linkage. The method produced small (0.5mg/harvest) amounts of a full TCR chain which necessitated the cleavage from the surface of the transfected cells by a phosphatidyl inositol-specific phospholipase C (Lin, A.Y., 1990, *Science*, 249:677-679).

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Others have produced chimeric molecules by shuffling the variable and constant domains of the murine TCR with the constant region of an immunoglobulin κ light chain. The investigators were able to achieve small amounts of a secreted soluble form of a heterodimeric αß TCR (Gregoire, C., et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8077-8081). Gregoire et al. show a murine chimera consisting of the Cα and Vα genes of the KB5-C2 joined to the C region of the κ light chain of the S105 monoclonal antibody. Also shown is a VßCßCκ chimera. Both are transfected into a mammalian B cell myeloma that does not express native immunoglobulin heavy or light chains. (See also, Weber, S., et al., 1992, *Nature*, 356:793-795).

Novotny et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8646-8650, demonstrate a method for producing soluble TCRs with antigen-combining properties. A synthetic oligo linker was cloned onto the C terminus of the Vß and the N terminus of the Vα region resulting in a single-chain VßVα. The resultant expression vector encodes the pelB leader followed immediately by in frame Vß, the linker, and the Vα segments. The expression of this construct is under the control of the lacZ promoter and is therefore IPTG-inducible.

Recently, one group has shown the secretion of a small amount of a T cell receptor V region fused to a carboxyl-terminal poyhistidine "tail". The plasmids contain the pelB leader and are under the control of a chemically inducible promoter (Ward, E.S., 1991, *Scand. J. Immunol.*, 34:215-220). The work fails, however, to describe how to obtain a T cell antigen receptor V region protein in the absence of a fusion partner, or how to obtain commercially useful amounts of the receptor V region.

There is a great need, therefore, for agents and pharmaceutical compositions which have the properties of specificity for the targeted autoimmune response, predictability in their selection, and convenience and reproducibility of preparation.

SUMMARY OF THE INVENTION

The present invention addresses the above-mentioned problems by providing for the first time the successful production of a universal agent for treating a given T cell-mediated disease in which T cell subsets are implicated. In particular, the invention provides useful quantities of full length TCR V region proteins that may be naturally processed, providing for multiple active fragments upon *in vivo* processing, and presented by antigen-presenting cells of each individual, thereby eliminating the need for "tailor-made" therapeutic agents. Also provided is a class of recombinant expression vectors for the production of TCR V region proteins in bacteria. The recombinant TCR V region proteins of the instant invention are useful in the treatment, prevention or suppression of an immune-related disorder. In another embodiment, the TCR molecules can be combined in an admixture with a pharmaceutically acceptable carrier for use as a therapeutic composition. In another embodiment, the claimed

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TCR V region proteins are useful to generate anti-TCR antibodies. Alternatively, the TCR V region proteins can be used diagnostically to characterize a particular immune-related disorder. The TCR V region proteins of the instant invention can be used as diagnostic probes based their ability to bind with autoantibodies. It is an advantage of the present invention that the T cell antigen receptor V region proteins are expressed in the absence of fusion proteins or affinity tails. It is a further advantage of the present invention that the TCR V region proteins are produced in commercially acceptable amounts that enable the preparation of compositions which are therapeutically effective in treating T cell-mediated autoimmune disease.

The invention further provides for an expression vector for the production of a pure full length TCR V region molecule. The expression vectors of the invention include as an essential element a coding region for a T cell antigen receptor V region. The TCR coding region of the invention is any nucleic acid molecule encoding a full length TCR V region protein. In a preferred embodiment, the TCR coding region includes the structural coding sequence for the Vß5.3 region.

The expression vectors described herein provide a means for the inducible expression of TCR V region proteins. In preferred embodiments, the T cell antigen receptor V region coding sequence is under the transcriptional control of an inducible bacterial promoter. According to the present invention, the inducible bacterial promoter is subject to either chemical or thermal regulation. Most preferably, the inducible bacterial promoter is subject to thermal induction.

In one embodiment, the expression vector of the instant invention includes a regulatory gene coding for a protein capable of binding to the promoter and thereby repressing transcription from the inducible promoter. In a preferred embodiment, the promoter is derived from the *E. coli lac* operon and the regulatory gene comprises a functional mutation of the *E. coli lacI* repressor gene. This mutant *lacI* gene (*lacI*ts) codes for a temperature-sensitive repressor protein, and utilizing this *lacI*ts gene permits thermal induction of the promoter. In a preferred embodiment, the inducible bacterial promoter is a *trc* promoter, and the thermal induction is provided by the *lacI*ts regulatory gene.

In one embodiment, the regulatory gene is located on a cotransformed vector. In another embodiment, the regulatory gene is located in the bacterial host cell chromosome. In a preferred embodiment, the regulatory gene is located in the recombinant expression vector. In a further preferred embodiment, the transcription direction of the mutant *lacI* gene is in the same orientation as the T cell antigen receptor structural gene to be expressed.

The recombinant expression vectors of the present invention may advantageously include a coding sequence for a leader or signal peptide. Such a leader sequence, fused in frame and 5' to the TCR Vregion coding region to be expressed, directs the synthesis of a TCR V

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region protein having an N-terminal leader peptide which in turn directs secretion of the recombinant protein across the host cell membrane. Other embodiments of the present invention do not utilize a leader sequence, and the desired V region product is isolated from the cells, e.g., by urea extraction.

In a preferred embodiment, the recombinant expression vectors of the present invention comprise, in the 5' to 3' direction: a *trc* promoter, a ribosome binding site, an optional leader sequence, a coding region for a full length T cell antigen receptor V region, a stop codon, one or more transcription terminators, a *lacI*ts regulatory gene such that the transcription direction is the same as that of the coding region for the T cell antigen receptor V region, a bacterial origin of replication, and a tetracycline resistance gene.

This invention also provides methods for producing TCR V region proteins at significantly increased levels than previously reported.

The present invention provides compositions and methods for treating a patient having an immune-related disorder. Such treatment comprises administering to a subject in need of such treatment, a composition in accordance with the present invention, in an amount and for a period of time suitable to regulate said immune disorder. It is an advantage of the present invention that the compositions of the invention allow for treatment of an immune related disorder without the need to select a particular peptide fragment and without tailoring the particular composition to the particular individual to be treated.

The invention also relates to the use of a full length T cell antigen receptor V region protein in therapy for an autoimmune disease or disorder. In a specific embodiment, the invention relates to the use of a full length Vß5.3 protein in therapy. In another embodiment, the invention provides for the use of a full length T cell antigen receptor V region protein in the manufacture of a medicament for treating an autoimmune disease or disorder. Therefore, in one embodiment, the invention enables the use of a full length Vß5.3 protein in the manufacture of a medicament for the treatment of an immune related disease or disorder such as MS.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. The complete nucleotide sequence [SEQ ID NO. 25 and SEQ ID NO. 26] and amino acid sequence [SEQ ID NO. 27] of the full length Vß5.3 protein of the invention.

Figure 2. Plasmid map of pKTB-Vß5.3. This plasmid contains Ptrc, the *trc* promoter; g10, the g10 leader gene segment from bacteriophage T7; SD1, ribosome binding site 1; MC, a mini-cistron encoding ribosome binding site 2 (SD2) AAG GAG (see SEQ ID NO. 28 for complete mini-cistron nucleotide sequence) and the peptide Met Tyr Arg Leu Asn Lys Glu Glu (SEQ ID NO. 29); STII, the STII leader sequence; Vß5.3, the Vß5.3 coding region; T1T2,

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transcription terminators of the *rrnB* operon; the *lacI*ts gene encoding the temperature-sensitive *lac* repressor; Ori, the origin of replication and Tet^r, the tetracycline resistance gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes recombinant expression vectors and methods for the efficient production of large quantities of a T cell antigen receptor V region protein and the use thereof, including the diagnosis, treatment, prevention or suppression of immune-related diseases or disorders.

A series of vectors has been developed for the production of enhanced levels of a full length TCR V region protein. Additionally, a method is provided for producing commercially useful amounts of a full length recombinant TCR V region protein using an expression vector of the instant invention. The methods and expression vectors of the instant invention are unique because they provide for the production of a full length T cell antigen receptor V region in the absence of extraneous protein sequences or fusion partners. As detailed in the Background of the Invention, *supra*, T cell antigen receptor V regions have, in the past, been produced only as part of a larger protein, and at very low levels. These extraneous proteins may cause undesirable immunogenicity when used *in vivo*, and they are frequently cited by regulatory authorities as impermissible impurities. Previously purified V region proteins included other regions of the TCR, such as the C region, fused to the V region. Others constructed expression systems that provided for a TCR V region as a chimeric protein, e.g., a V region fused to a portion of an immunoglobulin molecule.

The expression vectors and methods of the instant invention further provide for enhanced levels of expression of a T cell antigen receptor V region protein. A primary consideration in the production of a protein that has commercial utility, for example, in research, diagnostics and therapeutics, is the manufacture of commercially useful amounts of a protein. The present invention therefore provides for the production of enhanced levels of TCR V region proteins. The present invention makes possible the production of V region protein in large amounts, e.g., from 1 and 500 mg/liter of bacterial culture, or more. Preferably, the production level of the TCR V region protein is at least 100 mg/liter. In a most preferred embodiment, the instant invention provides for the production of at least 300 mg of Vβ protein per liter of bacterial cell culture.

The methods and expression vectors of the instant invention provide for the production of a TCR V region protein in the absence of extraneous protein sequences. The invention therefore eliminates several steps associated with obtaining a purified TCR V region protein if prepared according to the prior art. In one embodiment, the present invention provides for

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increased expression of V region proteins by thermal induction, which avoids the necessity of eliminating chemical inducers, such as IPTG.

Because TCR V region proteins are produced according to the invention in the absence of extraneous proteins, the invention provides for simplified production of relatively pure product. The invention eliminates additional processing steps, such as the introduction of enzymes or chemical reagents into the system to obtain a protein free of any fused non-TCR peptide segments or affinity tails. Not only are such manipulations likely to reduce the amount of V region product recovered, they also introduce elements which must be eliminated, i.e., by additional purification steps.

1. T CELL ANTIGEN RECEPTOR V REGION

The T cell antigen receptor V region protein of the instant invention is any full length T cell antigen receptor V region. A full length TCR V region protein is encoded by a coding region for a T cell antigen receptor V region. The TCRV region coding region is a nucleotide sequence encoding a full length T cell antigen receptor V region protein. Within the scope of the present invention, V region includes the variable region of the T cell antigen receptor. In its broadest aspects, the teachings of the present invention may be applied to obtain enhanced expression of TCR proteins including the V region and, additionally, all or part of the D and/or J regions.

TCR V region protein is produced in the absence of extraneous protein sequences, such as, for instance, other TCR regions, such as the constant region, the transmembrane region or the cytoplasmic region. Further, the TCR V region protein of the invention is produced in the absence of non-TCR fusion partners, such as affinity tails. Further, the TCR V region protein of the invention is not produced as a part of a larger chimeric protein including portions of other protein molecules, such as an immunoglobulin protein.

Recognizing that the DNA sequence encoding one of the complementarity-determining regions (CDRs) of a T cell receptor's V region may actually span the known juncture between the V and D coding regions (as in the case of the CDR3 of Vβ 5.3), as used herein, a "full length" V region coding region may include the entire coding sequence of such a CDR that spans the V-D juncture, the portion of such a CDR that only resides in the known V region, or none of the sequence coding for such a CDR. Furthermore, the full length T cell antigen receptor V region protein of the present invention can be any of the T cell antigen receptor V regions and is not limited to only Vβ regions (i.e., V regions of the TCR β-chain). Therefore, the T cell antigen receptor V region protein can be a variable region of the α subunit, the β subunit, the δ subunit, or the γ subunit.

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In a preferred embodiment, the TCR V region protein of the instant invention is any T cell antigen receptor V region for which a full length coding sequence is available. Methods of obtaining the full length coding sequences of the instant invention are well known in the art. Human V region domain DNA sequences can be isolated in accordance with well known procedures from a variety of human cells. Preferably, the cells are peripheral blood T lymphocytes.

The DNA sequences of the present invention may be prepared in various ways. It is preferred that the DNA sequences of the T cell antigen receptor V regions be prepared by polymerase chain reaction (PCR) using DNA sequences coding for the intact TCR as templates. A human Vβ region can be prepared, for instance, via amplification of cDNA prepared from human peripheral blood lymphocytes stimulated by an anti-CD3 antibody, following the procedure of Choi et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:8941-8945.

In one aspect of the present invention, the coding region for a full length T cell antigen receptor V region is obtained from any clone containing the full length sequence. For example, several TCR V region sequences and cDNA clones containing the full length coding regions are described in Concannon et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:6598-6602.

Preferred TCR V region proteins of the instant invention are those implicated in an immune-related disease or disorder, particularly, e.g., Vβ 8.1 (Kawasaki disease; see, Abe et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:4066), Vβ 6.1 (leprosy; see, Wang, et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:188), Vβ5.1 (Lyme disease; see, Lahesma et al., 1993, *J. Immunol.*, 150:4125), Vβ5.2, or Vβ5.3, or Vβ6.1 (multiple sclerosis; see Kotzin, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:9161), Vβ2.1, or Vβ3.1 (rheumatoid arthritis; see Uematsu, et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8534). (See also, Hafler, D.A. et al., 1988, *J. Exp. Med.*, 167:1313; and Mantgazza R. et. al., 1990, *Autoimmunity*, 3:431).

The TCR expressed by a T cell clone in an autoimmune disease or a T cell clone responding to a particular autoantigen can be identified using TCR-specific antibodies, for example polyclonal, monoclonal or chimeric antibodies that are specific for a TCR variable segment, in conjunction with an antibody labelling method, including fluorescence microscopy, flow cytometry, immunocytochemistry, or other techniques known in the art. Such antibodies have been described for a number of TCR α and/or β chain V regions (see, for example, International Patent Publication WO 90/06758).

Alternatively, the DNA or mRNA of the T cell clone can be probed directly, or after amplification by PCR (Synha et al., 1988, *Science*, 239:1026; Saiki et al.,1986, *Nature*, 324:163), and specific hybridization with nucleic acid probes for the various TCR gene families,

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using hybridization methods well known in the art. The TCR sequence, or a part thereof, can then be obtained directly from the amplified, rearranged DNA or mRNA.

Expression of a particular TCR can also be identified by determining the nucleic acid sequence encoding at least part of the TCR, for example, after cloning the TCR V gene, or by determining the amino acid sequence of at least part of a TCR protein. It will be apparent that any of the above-mentioned approaches, or additional approaches known to one of skill in the art, will result in the identification of the TCR expressed on a T cell or clone or line of T cells.

Examples of molecular approaches used to correlate TCR gene expression with disease include:

(1) producing and analyzing cDNA libraries obtained from the disease-related T cells obtained from one or more subjects having the disease, to determine the presence of frequently used or 'dominant' TCR genes;

(2) Southern analysis of disease samples to determine whether specific genetic polymorphism (e.g., RFLPs) or oligoclonal TCR rearrangements exist;

(3) analysis of disease samples by cDNA synthesis, PCR amplification, and slot blot hybridization procedures; and

(4) *in situ* nucleic acid hybridization of TCR probes to T cells without prior culture of these cells.

Preferred TCR V region proteins include Vβ8.1, Vβ5.2, or Vβ5.3. Double-stranded cDNA encoding Vβ5.3 and the amino acid sequence for Vβ5.3 are shown in Figure 1. (See, SEQ ID NOS. 25, 26 and 27.)

A T cell antigen receptor V region clone can be introduced into the expression vectors of the instant invention by methods known to those skilled in the art. For example, from a cDNA clone, the V region is PCR synthesized incorporating an initiating ATG at the 5' terminus. Any convenient restriction site can be synthesized which incorporates an initiating codon and reconstitutes the 5' coding region of the V region. In a preferred embodiment, the restriction site is an *Nco*I site. A stop codon and an appropriate restriction site are engineered at the 3' end of the V region coding sequence so that it can be subcloned into the vector.

It should be understood that the methodology of the present invention can be used to prepare full length TCR V regions derived from animal species other than human.

2. EXPRESSION VECTORS

A recombinant expression vector within the scope of the present invention is a plasmid which contains exogenous DNA in the form of a nucleic acid coding TCR V region to be expressed. The recombinant expression vector of the present invention is introduced into a

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bacterial host cell for example by transformation, and transformants are isolated and cloned which express the desired V region protein.

The present invention concerns expression vectors containing the DNA sequence coding for the full length TCR V region protein. Preferably, the TCR V region proteins are those T cell antigen receptor proteins specifically described herein. An expression vector within the scope of the present invention is a plasmid which contains the TCR V region gene to be expressed under the transcriptional and translational control of regulatory elements, such as for instance the promoter, ribosomal binding site and transcription terminator. In addition it contains an origin of replication for stable replication in bacteria. It also preferably includes a gene coding for resistance to an antibiotic to facilitate selection of plasmid-bearing transformant cells. Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell.

Expression vectors useful in the present invention are often in the form of plasmids. Plasmids refer to circular double-stranded DNA sequences which are not integrated in the chromosome. The expression vectors of the present invention may also include other DNA sequences, for example leader or signal sequences which provide for targeting or stability of the expression product, and/or regulatory sequences which allow modulation of gene expression, for example in response to a shift in temperature or a change in composition of the growth medium.

The expression vectors contain one or more control sequences operably linked to the DNA sequences coding for the TCR V region. The term "operably linked" within the scope of the present invention means that one nucleic acid sequence ,which has a regulatory function for gene expression or encodes a functional protein domain, is covalently linked, either 5' or 3', to a second nucleic acid sequence encoding a protein, so that the regulatory function is exerted over the second nucleic acid sequence or the encoded functional domain will be fused in frame to the protein encoded by the second nucleic acid. Such regulatory functions for gene expression typically include initiation of transcription, termination of transcription, and mRNA transcript processing. Nucleic acid sequences that exert such regulatory functions over gene expression include promoters of transcription, transcription terminators, and mRNA processing signals. Functional domains that may be "operably linked" to another protein include, for example, leader or signal peptides which permit transport of proteins across cell membranes.

In one embodiment, the promoter may be any inducible promoter. In a preferred embodiment, the promoter is one that is regulated by the presence or absence of certain media components. One skilled in the art will recognize that several bacterial promoters subject to the control of the presence or absence of certain media components can be used in connection with the expression vectors of the instant invention. It is preferred that the promoter be a strong

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bacterial promoter such as the *lac* promoter, the *tac* promoter, and the *trc* promoter. Therefore, in one embodiment, a promoter for a TCR V region coding sequence in a vector of this invention is subject to regulation by the presence or absence of isopropyl-ß-D-thiogalactopyranoside (IPTG), a gratuitous chemical inducer molecule of the *lac*, *tac*, and *trc* promoters. In preferred aspects, the IPTG-inducible promoter is the *lac* promoter or the *tac* promoter. In a more preferred embodiment, the IPTG-inducible promoter is the *trc* promoter (Brosius et al., 1985, *J. Biol. Chem.*, 260:3539-3541) which is similar to the *tac* promotor derived by fusing the -10 region of the *lac* UV5 promoter with the -35 region of the tryptophan operon (de Boer et al., 1983, *Proc. Natl. Acad. Sci. USA*, 80:21-25; Amann et al., 1983, *Gene*, 25:167-178.) The *trc* promoter is identical to the *tac* promoter with the exception of a single base pair change in the promoter (Brosius et al., (1985) *supra*).

In a further embodiment, the expression vector of the instant invention is modified to allow for the secreted expression of the full length TCR V region protein of the invention by a temperature shift. According to this embodiment, the expression vector allows for the expression of TCR V region protein in the absence of chemical inducers, which obviates the need to use such elements as IPTG and bacterial host strains containing the *lacI* or the *lacI*^{q} genes. Preferred embodiments of the expression vectors of the instant invention include a regulatory gene which allows for the thermal induction of protein expression. The regulatory gene sequence most preferably is the *lacI*ts gene, which encodes a temperature-sensitive *lac* repressor.

In a further preferred embodiment, the *lacI*ts gene is located proximal to and downstream of the transcription terminator, and its transcription direction is the same as that of the V region coding sequence.

In addition, the expression plasmids of this invention preferably contain one or more selectable genetic marker(s). Preferably, the selectable genetic marker confers resistance to a antibiotic, such as ampicillin, kanamycin or tetracycline. Most preferably, the selectable marker is the tetracycline resistance gene.

Additionally, the vector includes a bacterial origin of replication. In a preferred embodiment, the origin of replication is derived from pBR322. The expression vector also contains a ribosome binding site and the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118.)

In a preferred embodiment, an expression vector of the instant invention contains as essential elements in the 5' to 3' direction: a DNA sequence which contains a promoter and an operator; a DNA sequence which contains a ribosomal binding site for rendering the mRNA of the desired gene capable of binding to ribosomes within the host cell; an ATG initiation codon

or a DNA sequence which is converted into an ATG initiation codon upon insertion of the desired gene into the vector; a restriction site for the insertion of the desired gene in frame with the ATG initiation codon or a multiple cloning site containing several restriction sites for the insertion of the appropriate gene or coding region to be expressed; a nucleic acid sequence encoding the T cell antigen receptor V region to be expressed; and a transcription terminator which serves to efficiently terminate transcription. The vector also contains a DNA sequence which contains an origin of replication from a bacterial plasmid capable of autonomous replication in the host cell and a DNA sequence which contains a gene associated with a selectable or identifiable phenotypic trait which is manifested when the vector is present in the host. The expression vector may optionally include a signal or leader coding sequence operably linked, i.e., fused in frame, to the 5' end of the nucleic acid sequence encoding the TCR V region so that translation of the leader sequence-TCR V region sequence results in a leader peptide-TCR V region fusion protein in which the leader peptide at the amino terminus of the fusion protein directs secretion of the TCR V region protein across a host cell membrane. In certain preferred embodiments, the expression vector contains a regulatory gene which allows for the expression of the secreted protein by a temperature shift.

In a more preferred embodiment, the expression vector contains the following essential elements in 5' to 3' direction: The *trc* promoter which is composed of the -10 region of the *lac* UV5 promoter fused with the -35 region of the tryptophan operon (de Boer et al., *supra*; Amann et al. (1983) *supra*); a strong ribosome binding site; the TCR V region coding region to be expressed; the two transcription terminators from the *rrnB* ribosomal RNA operon; the *lacI*ts gene coding for the temperature-sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. An example of such a preferred embodiment, the expression vector pKBi-Vβ5.3 was deposited under the Budapest Treaty with the American Type Culture Collection (ATCC) under accession no. 69739 on January 6, 1995.

Expression vectors of the invention may contain a leader sequence encoding a signal peptide operably linked 5' of the TCR V region coding region. On expression, amino-terminal signal peptide will direct secretion of the TCR V region protein across the host cell membrane, into the periplasm or the culture medium. One skilled in the art will recognize that many leader sequences may be used in connection with the expression vector of the instant invention. One suitable leader sequence is the pelB signal sequence from *E. carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383). Another suitable leader sequence is the heat stable enterotoxin II (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732).

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In one embodiment, the expression system contains the STII leader sequence, and the coding region for a V region is PCR synthesized to contain an appropriate restriction site at the 3' end and an engineered *Mlu*I site at the 5' end so that it can conveniently link to the *Mlu*I site incorporated into the 3' end of the STII leader. Therefore, in a further preferred embodiment, the expression vector comprises in the 5' to the 3' direction: the *trc* promoter which is composed of the -10 region of the *lac* UV5 promoter fused with the -35 region of the tryptophan operon; a strong ribosome binding site; the STII leader; the coding region for the T cell antigen receptor V region to be expressed; the transcription terminators from the *rrnB* operon; the *lacI*ts gene coding for the temperature sensitive *lac* repressor; the pBR322 origin of replication; and a tetracycline resistance gene. In a most preferred embodiment, the expression vector is pKB-Vβ5.3.

The expression vectors of the present invention may be constructed using standard techniques known in the art. (See, e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

The present invention additionally concerns host cells containing an expression vector which contains a DNA sequence coding for a full length TCR V region. Additionally, preferred host cells contain an expression vector comprising one or more control DNA sequences capable of directing the replication and or the expression of a DNA sequence coding for a full length T cell antigen receptor V region protein. Suitable host cells include prokaryotic host cells; and in preferred aspects, the prokaryotic host cell is a bacterial host cell, most preferably an *E. coli* cell.

One skilled in the art will understand from the teaching of the instant application that the particular expression vector used will influence the choice of host strains selected for the production of the full length TCR V region proteins. For instance, in the expression vectors subject to chemical induction within the scope of the present invention, bacterial host strains which harbor the *lacI* or *lacI*^{q} gene, which regulate the *trc* promoter, are especially useful. In expression vectors within the scope of the present invention that contain a regulatory gene such as the *lacI*ts gene, which provides for a temperature-sensitive repressor, bacterial host strains that do not harbor the wild type *lacI* or *lacI*^{q} genes are preferred. A particularly preferred host cell is LJ24.

In certain embodiments, the expression vector of the instant invention does not contain a leader sequence. In certain preferred embodiments the bacterial host strain is *E. coli* BL21, or more preferably a protease deficient *E. coli* strain, such as SG21163, SG22094, or SG21173.

The expression vectors of the present invention may be introduced into the host cells by various methods known in the art which may vary depending on the type of cellular host. For example, calcium chloride treatment or electroporation are commonly utilized for prokaryotic

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cells. (See, generally, Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).)

Once the expression vector has been introduced into an appropriate host cell, the host cell may be cultured under conditions permitting expression of large amounts of TCR V region.

Host cells containing an expression vector which contains a DNA sequence coding for a TCR V region protein may be identified by any appropriate means. In the following examples, for instance, production of V region protein is detected immunologically using an immunoassay. Alternatively, the host cell containing the expression vector of the instant invention may be identified by assessing the level of transcription of TCR V region DNA by measuring TCR-specific V region mRNA transcripts in the host cell (e.g., by Northern blot analysis). In yet another method, the host cell containing the expression vectors of the instant invention can be identified by DNA-DNA hybridization using probes complementary to the TCR V region coding sequence of the vector.

The DNA sequences of the present invention, expression vectors or DNA molecules may be determined by various methods known in the art. For example the dideoxy chain termination method as described in Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA.*, 74:5463-5467.

Following the examples provided herein and utilizing one or more of the detection methods described above, a person skilled in the art may, without undue experimentation, readily select the combinations of expression vectors and host cells that provide optimal expression of TCR V region proteins. By combining a particular host strain with a particular expression vector of this invention, it is possible to obtain relatively high level expression of full length T cell receptor protein. Preferably, the level of expression of full length TCR V region protein is at least 300 mg per liter of culture. Such levels of expression can be obtained, for example, by using a preferred embodiment in which *E. coli* SG21173 is the bacterial host strain and pKBi-Vβ5.3 is the expression vector (see examples, *infra*). Such combinations of host and expression vectors of this invention permit the production of significant quantities of full length T cell receptor protein at a lower cost than previously available.

Once expressed, the proteins of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity chromatography, column chromatography, gel electrophoresis and the like. (See. generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982).)

In a preferred embodiment, the T cell antigen receptor V region is soluble. By this it is meant that the T cell antigen receptor V region is soluble in an aqueous system. A "soluble" protein is generally characterized by its failure to sediment at room temperature under high G-

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force centrifugation (100,000 x g) out of an aqueous buffer. Solubility is particularly important in providing simplicity in purification of the TCR V region protein from the producing cell. Aqueous buffers used in protein chemistry typically have a buffering compound for maintaining a particular pH (typically within a physiological range of about pH 5-9), provide a particular ionic strength (typically between about 10 mM and about 500 mM), and often include a protease inhibitor and a mild detergent. Examples of standard buffers are phosphate buffered saline, Tris-buffered saline or any of a number of buffers used in protein isolation. (See, generally, *Methods in Enzymology*; Mahler and Cordes Biological Chemistry (2d Ed.), Harper and Row, New York (1966).)

3. USE OF T CELL ANTIGEN RECEPTOR V REGION PROTEINS

The present invention provides for the first time the means for efficiently and economically producing diagnostically, therapeutically and investigationally useful quantities of a TCR V region protein that is not a fusion protein or linked to an extraneous sequence, such as an affinity tail.

A. Investigational use and diagnostic use

The TCR V region proteins of the present invention are useful in structural and functional studies on the T cell antigen receptor (TCR). Besides serving as substrates or binding domains for specific forms of TCRs, these proteins may serve as tools for conformational studies, e.g., by approximating the native conformation of various portions of the T cell receptor.

The T cell V region proteins can also be used as diagnostic probes. In one specific embodiment, the TCR V region proteins of the present invention may be used in determining the amount or the presence of a certain TCR V region subfamily in a biological sample. One such assay is described in Rittershaus C.W., International Patent Publication WO92/08981, entitled "Therapeutic and Diagnostic Methods Using Total Leukocyte Surface Antigens". As such, the present invention provides methods for diagnosing an immune-related disease, such as multiple sclerosis (MS), by detecting the specific subset of T cells associated with the disease.

In another embodiment, the present invention can be used in the generation of significant quantities of T cell antigen receptor V region proteins for use in antibody production. The antibodies can be prepared by using any of a number of techniques well known in the art. For producing a monoclonal antibody (MAb), any method which provides for the production of antibody molecules by continuous cell lines in culture may be used. These methods include, but are not limited to, the hybridoma techniques originally described by Kohler and Milstein, 1975, *Nature*, 256:495-497); the more recent human B cell hybridoma technique (Kozbor et al., 1983, *Immunology Today*, 4:72); the EBV technique (Cole et al., Monoclonal Antibodies and Cancer

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Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)); and trauma techniques. For a review of antibody production methods see: Hartlaw, E., et al., Antibodies: A laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

The TCR Vregion proteins of the invention can be used as diagnostic probes based on their ability to react with autoantibodies present in a biological sample.

The present invention also contemplates labeled derivatives of the TCR V region proteins which can be detected by appropriate monitors, such as scintillation and Geiger counters, or radiographic film. Such derivatives include, for example ^{125}I, ^{131}I, ^{14}C, ^{35}S, ^{3}H, ^{112}In, ^{99}M, Tc and the like, for *in vitro* and *in vivo* diagnostic purposes.

B. Therapeutic use

As mentioned above, the present invention is useful in the therapy of immune-related disease. The term "immune-related disease" as used herein refers to a disease in which the immune system is involved in the pathogenesis of the disease, or in which appropriate stimulation of the immune system can result in protection from the disease. Relevant diseases include, but are not limited to, autoimmune diseases, neoplastic diseases, infectious diseases, hypersensitivity, transplantation, graft-versus-host disease, and degenerative nervous system diseases. Autoimmune diseases include, but are not limited to, arthritis, such as rheumatoid arthritis, type I diabetes, juvenile diabetes, multiple sclerosis, autoimmune thyroiditis (Hashimoto's thyroiditis), myasthenia gravis, systemic lupus erythematosus (SLE), Sjögren's syndrome, Graves disease, Addison's disease, Goodpasture's syndrome, scleroderma, dermatomyositis, myxedema, polymyositis, pernicious anemia, inflammatory bowel disease including Crohn's disease and autoimmune atrophic gastritis, and autoimmune hemolytic anemia. Neoplastic diseases include, but are not limited to, lymphoproliferative diseases such as leukemias, lymphomas, Non-Hodgkin's lymphoma, and Hodgkin's lymphoma, and cancers such as cancer of the breast, colon, lung, liver, pancreas, melanoma, renal carcinoma, etc. Infectious diseases include but are not limited to viral infections caused by viruses such as HIV, HSV, EBV, CMB, Influenza, Hepatitis A, B, or C; fungal infections such as those caused by the yeast genus *Candida*; parasitic infections such as those caused by schistosomes, filaria, nematodes, trichinosis or protozoa such as trypanosomes causing sleeping sickness, plasmodium causing malaria or leishmania causing leishmaniasis; and bacterial infections such as those caused by mycobacterium, corynebacterium, or staphylococcus. Hypersensitivity diseases include but are not limited to Type I hypersensitivities such as contact with allergens that lead to allergies, Type II hypersensitivities such as those present in Goodpasture's syndrome, and myasthenia gravis.

Therapeutic use of the TCR V region proteins of the invention is premised upon the correlation between a specific immune-related disease and the preferential expression of a

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particular T cell antigen receptor V region protein or the expanded usage of a particular sequence encoding a TCR V region protein. Though the present invention should not be limited by any particular scientific theory, the TCR V regions are useful in part because they may be used to regulate the immune response in an individual. Specifically, where the presence or abnormally high expression of a particular V region correlates with particular immune-related disorder, one can treat the individual by neutralizing the T cells carrying that particular V region protein. Further, introduction of the TCR V region protein may affect immunosuppressive functions by stimulating regulatory T cells which will specifically interact with T cells bearing a targeted V region. It is also likely that the TCR V region protein may elicit an antibody response that will specifically interact with the targeted V region on T cells and in turn modulate the immune response.

The full length T cell antigen receptor V region proteins of the instant invention provide distinct advantages in the treatment of an immune related disease. Prior to the instant invention, therapeutic options for treatment of immune related disease with specific T cell involvement included: (a) no treatment with possible spontaneous resolution; (b) treatment with peptides corresponding to distinct segments of the T cell antigen receptor V region; (c) nonspecific treatments such as steroids or non-steroidal anti-inflammatory agents. However, none of these afforded the particular advantage of the present invention. The therapeutic methods of the instant invention have the distinct advantage over the prior art methods of treating an immune related disease in that the methods of the present invention target only the particular T cell subset expressing a particular TCR V region and can be used across a diverse range of affected individuals, since the invention provides for the elimination of the need to select a particular peptide based on the MHC haplotype of a particular individual. The method of the present invention more closely approaches the goal of modulating only disease-related T cells while sparing or not affecting other T cells in the subject. The invention therefore achieves a greater specificity of therapy across a wider population.

In a preferred embodiment, a full length T cell antigen receptor V region protein may be used to down-regulate or eliminate a subset of T cells that exhibit a TCR incorporating the same V region. The T cell antigen receptor V region protein can be used in a therapeutic situation to modulate the activity of those T cells bearing the targeted V region or to induce unresponsiveness in the actively responding population of T cells bearing the targeted V region. In this situation the T cell antigen receptor is used as a therapeutic composition in a subject.

Specific T cell antigen receptor V region proteins associated with a given disease are identified using any of a variety of techniques well known in the art. A genetic approach using

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patients known to have myasthenia gravis or multiple sclerosis was described by Oksenberg, J.R., et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:988-992.

The term treatment in the instant invention is meant to include the situations of "prevention," "suppression" or "treatment" of the disease. "Prevention" involves administration of the protective composition prior to the induction of the disease. Thus, for example, in the animal model of experimental autoimmune encephalomyelitis, EAE, successful administration of a protective composition prior to injection of the encephalitogen that induces the disease resulted in "prevention" of the disease.

"Suppression" involves administration of the composition after the inductive event but prior to the clinical appearance of the disease. Again, using the EAE example, successful administration of a protective composition after injection of the encephalitogen, but prior to the appearance of neurological symptoms, resulted in "suppression" of the disease.

"Treatment" involves administration of the protective composition after the appearance of the disease. In the EAE example, successful administration of a protective composition after injection of encephalitogen and after clinical signs had developed resulted in "treatment" of the disease.

For measuring preventative, suppressive or therapeutic benefit of the TCR V region protein of the present invention, certain clinical outcomes are measured. In particular, alteration of proliferative response of lymphocytes *in vitro* can be measured. Assays for measuring the therapeutic benefit of the particular V region protein are known in the art.

Animal model systems which can be used to screen the effectiveness of the proteins in protecting against or treating the disease are available. Systemic lupus erythematosus (SLE) is tested in susceptible mice as disclosed by Knight et al., 1978, *J.Exp. Med.*, 147:1653 and Reinersten et al., 1978, 299:515. Myasthenia gravis (MG) is tested in SJL/J female mice by inducing the disease with soluble AChR protein from another species as described in Lindstrom et al., 1988, *Adv. Immunol.*, 42:233-284. Arthritis is induced in a susceptible strain of mice by injection of Type II collagen as described by Stuart et al., 1984, *Ann. Rev. Immunol.*, 42:233-284. Adjuvant arthritis is induced in susceptible rats by injection of Mycobacterial heat shock protein as described by Van Eden et al., 1988, *Nature*, 331:171-173. Thyroiditis is induced in mice by administration of thyroglobulin as described by Maron et al., 1980, 152:1115-1120. Insulin-dependant diabetes mellitus (IDDM) occurs naturally or can be induced in certain strains of mice such as those described by Kanasawa, et al., (1984). EAE in mice and rats serves as a model for MS in humans. In this model, the demyelinating disease is induced by administration of myelin basic protein (MBP) as described by Paterson, P.Y., Textbook of Immunopathology,

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(Mischer et al., eds.), Grune and Stratton, New York, pp. 179-213 (1986); McFarlin, D.E. et al., 1973, *Science*, 179:478-480: and Satoh, J., et al., 1987, *J.Immunol.*, 138:179-184.

Of course, the identical proteins may not be effective in humans since they may not correspond to an appropriate site of the disease-associated human TCR. It is to be understood that modifications of the protein sequence delineated in a particular animal model may be required in order to treat subjects belonging to other species, including humans.

4. PHARMACEUTICAL COMPOSITIONS CONTAINING THE T CELL ANTIGEN RECEPTOR V REGION

The proteins and compositions of the present invention, or their functional derivatives, are well suited for the preparation of pharmaceutical compositions. The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compositions of the invention. Foremost among such animals are humans, although the invention is not intended to be so limited.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The proteins and pharmaceutical compositions can be administered parenterally by bolus injection or by gradual perfusion over time.

The dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The dose ranges for the administration of the compositions of the present invention are those large enough to produce the desired effect, whereby, for example, an immune response to the proteins as measured by delayed-type hypersensitivity (DTH) or antibody production, is achieved, and the immune-related disease is significantly prevented, suppressed, or treated. The doses should not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Preferred doses for humans range between about 0.001 - 1 mg/kg body weight.

In addition to proteins of the invention which themselves are pharmacologically active, pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferred compositions include the inclusion of an adjuvant, such as alum, or other adjuvants known in the art. (See, for example, Warren et al., 1986, *Ann. Rev. Immunol.*, 4:369-388; Chedid, L., 1986, *Feder. Proc.*, 45:2531-2560.)

To enhance delivery or bioactivity, the proteins can be incorporated into liposomes using methods and compounds known in the art.

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Preparations which can be administered orally in the form of tablets and capsules, preparations which can be administered rectally, such as suppositories, and preparations in the form of solutions for injection or oral introduction, contain about 0.001 to about 99 percent, preferably from about 0.01 to about 95 percent of active compound(s), together with the excipient.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the proteins in water-soluble form, for example, water-soluble salts. In addition, suspensions of the proteins as appropriate oil injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions containing substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The proteins are formulated using conventional pharmaceutically acceptable parenteral vehicles for administration by injection. These vehicles are nontoxic and therapeutic, and a number of formulations are set forth in Remington's Pharmaceutical Sciences (Martin, E.W., ed.) Mack Publishing Co. Easton, PA (1990). Nonlimiting examples of excipients are water,

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saline, Ringer's solution, dextrose solution and Hank's balanced salt solution. Formulations according to the invention may also contain minor amounts of additives such as substances that maintain isotonicity, physiological pH, and stability.

The proteins of the invention are preferably formulated in purified form substantially free of aggregates and other protein materials, preferably at concentrations of about 1.0 ng/ml to 100 mg/ml.

Effective doses of the proteins of this invention for use in preventing, suppressing, or treating an immune-related disease are in the range of about 1 ng to 100 mg/kg body weight. A preferred dose range is between about 10 ng and 10 mg/kg body weight. A more preferred dose range between about 100 ng and 1 mg/kg body weight.

Methods to increase the effectiveness of proteins are known in the art. These techniques may be used in conjunction with the proteins of the instant invention.

The following examples are offered by way of illustration and not limitation.

EXAMPLES

The following general techniques are used throughout the following Examples.

1. GENERAL MATERIALS AND METHODS.

A. Bacteria, plasmids, and general methods

Escherichia coli GM2929, methylase-negative, and *E. coli* LJ24 (*lac* deletion) (Rasmussen et al., 1991, *J. Bacteriology*, 173:6390-6397) were gifts from Dr. Martin Marinus, University of Massachusetts Medical School, Worcester, MA. *E. coli* JM109 (Yanisch-Perron et al., 1985, *Gene*, 33:103-119) were purchased from Promega Corporation, Madison, WI (USA). BL21 (lon^-, $ompT^-$) (Wood, W.B., 1966, *J. Mol. Biol.*, 16:118-133; Studier and Moffatt, 1986, *J. Mol. Biol.*, 189:113-130) were purchased from Novagen, Madison, WI (USA). SG22094 (lon^-, clp^-), SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-) were gifts from Dr. Susan Gottesman, National Institutes of Health, Bethesda, MD (USA). These Gottesman strains have the *lacI* gene deleted. Bacteria were made competent for transformation by the method of Morrison, 1979, *Meth. Enzymology*, 68:326-331) or were purchased (DH5α) from GibcoBRL, Gaithersburg, MD (USA).

pKK233-2 (Amann and Brosius, 1985, *Gene*, 40:183-190) was purchased from Pharmacia, Piscataway, NJ (USA). pBR322 (Bolivar et al., 1977, *Gene*, 2:95-113) was obtained from New England Biolabs, Beverly, MA (USA). pBluescript II KS+ (Alting-Mees et al., 1992, *Meth. Enzymology*, 216:483-495) was obtained from Stratagene, La Jolla, CA (USA). pDC952 containing the *argU* (*dnaY*) gene (Lindsey et al., 1989, *J. Bacteriology*, 171:6197-6205) was a gift from Dr. James R. Walker, University of Texas, Austin, TX. pSE420 (Brosius,

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1992, *Meth. Enzymology*, 216:469-483) and pKK480-3 (Brosius, (1992) ~~*supra*) were gifts from~~ Dr. Jürgen Brosius, Mount Sinai School of Medicine, New York, NY. pUCts (Bukrinsky et al., 1988, *Gene*, 70:415-417) was obtained from Michail I. Bukrinsky via Jürgen Brosius.

Plasmid constructions were performed by standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York(1989)). DNA restriction and modification enzymes were purchased from New England Biolabs, unless otherwise stated, and were used according to the manufacturer's specifications. Synthetic oligonucleotides were purchased from Operon Technologies, Inc., Alameda, CA (USA), unless otherwise indicated.

Oligonucleotides were annealed together by mixing equimolar concentrations of the complementary oligonucleotides in 10 mM Tris pH8.0, 5 mM $MgCl_2$ and placing in 100°C for 5 min and then cooling slowly to 25°C. Polymerase chain reaction (PCR) was performed (Saiki et al., 1988, *Science*, 239:487-491) in a Perkin Elmer Cetus DNA Thermal Cycler, using the GeneAmp reagent kit (The Perkin-Elmer Corporation, Norwalk, CT (USA)). DNA sequencing employed the dideoxynucleotide chain termination method (Sanger et al., 1977, *Proc. Natl. Acad. Sci. USA*, 74:5463-5467) using the Sequenase kit (U.S. Biochemical, Cleveland, OH (USA)). All chemical reagents were of the highest purity available.

B. Growth and induction of cell cultures

For chemically induced cultures, frozen glycerol stocks were used to inoculate 5-25 ml 2XYT medium (16 g Bacto-Tryptone (Difco 0123), 10 g Bacto-Yeast Extract (Difco 0127), 10 g NaCl, pH 7.2-7.4, per liter water) containing 6 µg/ml tetracycline, and allowed to grow overnight at 37°C, 280 rpm. Typically, 1/100 volumes of the overnight culture were used to inoculate each of two 125-ml flasks containing 25 ml 2XYT medium plus 6 µg/ml tetracycline. Cultures were grown at 37°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units, and then induced with 1 mM isopropyl ß-D-thiogalactopyranoside (IPTG), and allowed to grow for a further 2 hr.

For thermal induction, cultures were grown at 30°C, 280 rpm until A_{580} was 0.5-1.0 absorbance units. Thereafter, one culture was maintained at 30°C, and the other was induced by transferring to a water bath at 42°C, and allowed to grow for a further 2 hr. Alternatively, a culture was induced by growing at 30°C until A_{580} was 0.3-0.6 absorbance units, and thereafter was divided into two aliquots; one was maintained at 30°C, and the other was allowed to grow at 42°C.

To assay for results of induction, duplicate samples equivalent to 1 ml of cells at A_{580} = 1.0 were removed (e.g., assuming A_{580} = 3.0, then 333 µl culture was removed). The samples were centrifuged in an Eppendorf microcentrifuge at 14,000 x g, 1 min., the supernatants discarded, and the cell pellets were stored at -20°C.

C. Stability study

Bacteria were streaked to single colonies three successive times and a single colony from the third plate was used to inoculate 200 ml 2XYT medium containing 6 µg/ml tetracycline. The culture was grown to A_{580} = 0.5-1.0 absorbance units, and 60 ml were removed, mixed with 40 ml glycerol and 1 ml aliquots were distributed into 100 vials to be stored at -80°C. The remainder of the culture was divided into two aliquots; one was incubated uninduced, and the other was induced by IPTG addition or temperature shift. Both cultures were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed prior to and at 2 hr after induction, and assayed for A_{580} absorbance, pH, and protein production by EIA, acrylamide gel electrophoresis, and Western blotting.

Three vials from the frozen bank were tested in parallel for bacterial growth and protein production. Each vial (1 ml) was used to inoculate 200 ml 2XYT medium plus 6 µg/ml tetracycline, and incubated to A_{580} = 0.5-1.0 absorbance units. Thereafter each culture was divided into two aliquots, one to be incubated uninduced, the other to be induced chemically or thermally. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed from both cultures prior to and at 2 hr after induction and assayed as described above.

For the stability study, a 1-ml aliquot was removed from one of the three parallel cultures prior to induction, diluted 1/1000 in 2XYT medium, and 20 µl or 200 µl was inoculated into 200 ml 2XYT medium plus tetracycline, and allowed to grow to A_{580} = 0.5-1.0 absorbance units. Thereafter the culture was divided into two aliquots; one was induced, and both were allowed to grow for a further 2 hr. Samples equivalent to 1 ml culture at A_{580} = 1.0 were removed prior to and at 2 hr after induction and assayed as above.

D. Cell fractionation

The cells were pelleted by centrifugation at 7000 x *g*, 7 min, and the supernatant (culture medium) was recentrifuged at 14,000 x *g*, 5 min prior to electrophoresis. The cell pellet was resuspended in 20% (w/v) sucrose, 0.3 M Tris-HCl pH 8.0, 1 mM EDTA using 1/5 of initial culture volume, incubated at 25°C for 15 min, and centrifuged at 6000 x g, 7 min. The supernatant was discarded, and protein located in the periplasm was released by osmotic shock (Nossal and Heppel, 1966, *J. Biol. Chem.*, 241:3055-3062) by resuspending in 1/5 original volume cold water, and incubating at 0°C, 10 min. Following centrifugation at 9000 x *g*, 10 min, the supernatant was saved (periplasmic fraction); the cell pellet was resuspended in 1X TST (25 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, 10 ml/liter Tween 20), sonicated, centrifuged at 12,000 x *g*, 15 min, and the supernatant was saved (soluble cytosolic fraction).

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E. Polyacrylamide gel electrophoresis

The frozen *E. coli* cell pellet was resuspended in 70 µl water by vigorous vortexing or by sonication at 0°C (Sonic Dismembrator, Model 301, ARTEK Systems Corporation) three times each for 5 seconds, at power output 0.4. The disrupted cells were mixed with 70 µl 2X loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% ß-mercaptoethanol, 0.002% Bromophenol Blue) (Laemmli, 1970, *Nature*, 227:680-685.) Samples were incubated at 100°C, 5 min., centrifuged at 14,000 x *g*, 30 seconds, and 10-15 µl aliquots were loaded onto pre-cast 16% or 10-20% acrylamide Tricine gels (Novex, San Diego, CA (USA)) and run in reservoir buffer obtained from the same manufacturer. Electrophoresis was at 125 V constant voltage, or 45 mA constant current, per two gels, for about 2 hr, until the bromophenol blue tracking dye was off the gels. Markers were prestained low molecular weight protein standards (# SE130024, Integrated Separation Systems, Natick, MA (USA)) used at 1 µl per lane. Gels were stained overnight in 10% acetic acid, 25% methanol, 0.025% Coomassie Brilliant Blue R.

F. Western blot analysis

Following separation by SDS-PAGE, proteins were immunodetected (Towbin et al., 1979, *Proc. Natl. Acad. Sci. USA*, 76:4350-4354) by electrotransfer to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA (USA)) using a Semi-Dry electroblotter (Integrated Separation Systems). Proteins were transferred at 10 mA per cm^2 membrane for 1-3 hr, and the membranes were blocked overnight in blocking buffer (1X PBS, 2% dried milk, 0.1% Tween-20).

The membranes were washed three times, 5 min each in 1X PBS, 0.1% Tween-20, followed by washing three times, 5 min each in 1X PBS, and then they were submerged in an horseradish peroxidase (HRP)-conjugated monoclonal anti-Vβ5.3 antibody (13A2-HRP, 0.25 mg/ml) diluted 1:3000 in 1 X PBS, 0.1% Tween-20. Following gentle agitation for 2 hr, the membranes were washed as described above, and the proteins were visualized with the Enhanced Chemiluminescence assay (Amersham, Arlington Heights, IL (USA)) used according to the manufacturer's instructions. Film exposure was between 5 seconds and 1 minute.

G. Enzyme immunoassay

The frozen cell pellets were resuspended by vigorous vortexing in 20 µl 8 M urea, 50 mM Tris-HCl, pH 8.5, (Marston and Hartley, 1990, *Meth. Enzymology*, 182:264-276) and Vβ5.3 was quantified by enzyme immunoassay as follows:

Nunc-Immuno Maxisorp plates (VWR #62409-002) were coated with 100 µl/well 4C2 IgG at 4 µg/ml in PBS, and stored at 4°C overnight. The coating solution was discarded, and blocking buffer (1% casein hydrolysate in PBS, 0.05% Tween-20) was added at 200 µl/well. The plates were incubated at room temperature for 2 hr, 150 rpm, and then washed three times

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with wash buffer (PBS, 0.05% Tween-20). Samples (diluted 1/2,000 to ~~1/32,000~~) and Vβ5.3 standards in blocking buffer were added at 100 µl/well, and incubated at room temperature for 90 min, 150 rpm. The plates were washed three times with wash buffer, and an HRP-conjugated anti-Vβ5.3 monoclonal antibody (13A2-HRP) diluted 1/500 in blocking buffer plus 5% fetal bovine serum was added at 100 µl/well (0.5 µg/ml). The plates were incubated at room temperature for 90 min, 150 rpm, washed four times, and 100 µl/well O-Phenylenediamine (OPD) substrate was added. After 30 min, the reaction was terminated by adding 50 µl 2N H_2SO_4 per well. The absorbance was measured at 490 nm minus the background absorbance at 650 nm. Blank wells did not contain sample.

2. CONSTRUCTION OF PLASMIDS

EXAMPLE 1

Construction of pK2D

The starting point for the expression of the T cell antigen receptor V region proteins of the invention was the construction of a plasmid that contained the proper regulatory elements for expression of the T cell antigen receptor V region protein. These elements include the promoter, two transcription terminators, an origin of replication for stable replication in bacteria. Additionally because of regulatory concerns associated with the manufacture of a protein for which one possible indication is the *in vivo* use in a therapeutic setting, a gene coding for resistance to tetracycline was chosen to facilitate selection of plasmid bearing cells. Finally, the starting expression vector required a multiple cloning site, strategically engineered to facilitate the cloning of the preferred coding regions of the invention into the expression vector. The following example describes the unique starting plasmid of the invention.

The starting point for the construction of preferred expression vector was pKK233-2 (4.6 kb), described *infra*. The *Nco*I restriction site was converted to a *Spe*I site as follows: pKK233-2 was digested with *Nco*I, and the resulting 5' protruding ends were removed by incubation with mung bean nuclease at 25°C for 30 min. The plasmid was extracted twice with phenol-chloroform, precipitated with ethanol in the presence of 2 M ammonium acetate, and resuspended in water. Following dephosphorylation with bacterial alkaline phosphatase (GibcoBRL) the vector was ligated to a synthetic *Spe*I linker (#1085, New England Biolabs), and the ligation mix was transformed into DH5α competent cells to yield vector pKK233-2A.

Next, the tetracycline gene was restored as follows: pKK233-2A (4.6 kb) was digested with *Eco*RI and *Sal*I yielding two fragments, 279 bp and 4321 bp each. The two fragments were separated by agarose gel electrophoresis, and the large fragment was purified from agarose using the Geneclean Kit (BIO 101, La Jolla, CA (USA)). A 651-bp fragment containing the amino-terminal coding region of the tetracycline gene was excised with *Eco*RI and *Sal*I from

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pBR322, and ligated to the 4321-bp fragment from pKK233-2A. The resulting plasmid pK2B (4.97 kb) conferred tetracycline resistance on *E. coli* DH5α cells, and was further modified to delete a portion of the ß-lactamase gene and convert the *Hind*III site into a *Sac*II site, as follows:

A DNA segment spanning the unique *Spe*I site to the 3' terminus of the transcription terminators was PCR-synthesized using pK2B as template. The 5' "sense" primer contained restriction enzyme recognition sequences for *Spe*I, *Pst*I and *Sac*II in the 5' to 3' direction, underlined:

5' - TATAATGACTAGTCGCTGCAGCCAACCGCGGCTG -3' [SEQ ID NO 1]

The 3' "antisense" primer contained a *Sca*I site, underlined:

5' - GTCCTAGAGTACTGAGCGGATAC - 3' [SEQ ID NO 2]

1 ng of pK2B DNA template was mixed in a reaction volume containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 µg/ml gelatin, 200 µM each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), 1 µM each of the two primers, and 2.5 units AmpliTaq DNA polymerase. The final reaction volume was 100 µl, overlaid with 100 µl mineral oil. The DNA was denatured at 95°C for 2 min, and amplification was achieved with 30 cycles of 1 min at 95°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for primer extension. The amplified DNA was analyzed on 1% agarose gel run in 40 mM Tris-HCl pH 7.8, 5 mM NaAcetate, 1 mM EDTA.

The resulting 485-bp PCR-synthesized fragment was digested with *Spe*I and *Sca*I, and ligated to a 4129-bp fragment that was obtained by digestion of pK2B with *Spe*I and *Sca*I resulting in a plasmid called pK2C.

Plasmid pK2C (4.6 kb) was further modified to contain a multiple cloning site. First, pK2C was digested with *Spe*I and *Sac*II, and ligated to a double-stranded synthetic oligonucleotide containing 15 restriction sites. This multiple cloning site (MCS) was formed by annealing equimolar concentrations of two complementary synthetic oligonucleotides, 91 and 97 bp long, respectively, having the following sequences:

5' - GGCTCGAGCCTAGGCTGCAGCCCGGGGCGCGCGCGGCCGCAGGCC
TTTAATTAAGAGCTCCGGACCGCACAATGTGGGCGCGCCCTTAAGA - 3'
[SEQ ID NO 3]

5' - CTAGTCTTAAGGGCGCGCCCACATTGTGCGGTCCGGAGCTCTTAA
TTAAAGGCCTGCGGCCGCGCGCGCCCCGGGCTGCAGCCTAGGCTCGAG
CCGC - 3' [SEQ ID NO 4]

The MCS codes for the following restriction enzymes sites: *Spe*I, *Afl*II, *Asc*I, *Dra*III, *Rsr*II, *Sac*I, *Pac*I, *Stu*I, *Not*I, *BssH*II, *Xma*I, *Pst*I, *Avr*II, *Xho*I, and *Sac*II. The ligation mix was

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transformed into methylase-negative *E. coli* GM2929. The resulting plasmid pK2D (4.67 kb) was partially sequenced to ensure the integrity of the *in vitro* synthesized regions.

Results

The starting plasmid was pKK233-2 which contains the following elements: The *trc* promoter (Brosius et al., (1985) *supra*) which is similar to the *tac* promoter derived by fusing the -10 region of the *lac*UV5 promoter with the -35 region of the tryptophan promoter (de Boer et al., (1983) *supra*; Amann et al., (1983) *supra*). The plasmid also contains a ribosome binding site followed by a cloning site of three unique restriction enzymes (*Nco*I, *Pst*I and *Hind*III), followed by the transcription terminators of the *rrnB* ribosomal RNA operon of *E. coli* (Brosius et al., 1981, *Plasmid*, 6:112-118). In addition, pKK233-2 contains the ß-lactamase gene for ampicillin resistance, the pBR322 origin of replication, and a small non-functional carboxyl terminal fragment from the tetracycline resistance gene.

pKK233-2 was modified in order to destroy the *Nco*I site, restore the tetracycline resistance gene, delete a segment of the ß-lactamase coding region (thus inactivating the gene), and insert a multiple cloning site consisting of 15 restriction sites to produce pK2D.

EXAMPLE 2

Plasmid pK2D was further modified to incorporate either the pelB signal sequence from *Erwinia carotovora* (Lei et al., 1987, *J. Bacteriology*, 169:4379-4383) or the heat stable enterotoxin II (STII) signal sequence of *Escherichia coli* (Morioka-Fujimoto et al., 1991, *J. Biol. Chem.*, 266:1728-1732.)

Construction of pK-pelB.

Plasmid pK2D (4.67 kb) was engineered to include a ribosome binding site (Shine and Dalgarno, 1974, *Proc. Natl. Acad. Sci. USA*, 71:1342-1346; Shine and Dalgarno, 1975, *Nature*, 254:34-38; Steitz and Jakes, 1975, *Proc. Natl. Acad. Sci. USA*, 72:4734-4738) and the pelB signal sequence from *E. carotovora* (Lei et al., (1987) *supra*) to create plasmid pK-pelB (4.74 kb), as follows:

pK2D was digested with *Spe*I and *Sac*I located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 103 bp and 95 bp long, respectively, having the sequence shown below. A silent mutation (lower case letters) was introduced into the pelB sequence, in order to create an *Nco*I restriction site, underlined, facilitating the subsequent subcloning of the Vβ5.3 gene sequence.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAATACC
TATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCA
GCcATGGCCGAGCT - 3' [SEQ ID NO 5]

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5' - CGGCCATgGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCT
GCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGAAATAGA
ATTTA - 3' [SEQ ID NO 6]

Construction of pK-STII.

Plasmid pK2D was engineered to include a ribosome binding site and the heat-stable enterotoxin (STII) signal sequence of *E. coli* (Morioka-Fujimoto et al., (1991) *supra*), as follows:

pK2D was digested with *Spe*I and *Dra*III located in the MCS, and the vector was ligated to two complementary synthetic oligonucleotides, 105 bp and 98 bp long, respectively, having the sequence shown below. Two silent mutations (lower case letters) were introduced into the STII sequence, in order to create *Mlu*I and *Dra*III restriction sites , underlined, facilitating the subsequent subcloning of Vβ5.3.

5' - CTAGTAAATTCTATTTCAAGGAGACAGTCATAATGAAAAAGAA
TATAGCATTCCTACTAGCTTCAATGTTCGTCTTCTCTATTGCAACTA
ACGCgTACGCaCATT - 3' [SEQ ID NO 7]

5' - GtGCGTAcGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGCT
AGTAGGAATGCTATATTCTTTTTCATTATGACTGTCTCCTTGAAATA
GAATTTA - 3' [SEQ ID NO 8]

EXAMPLE 3

The plasmids containing the signal sequences from Example 2 *supra* were further modified to include two regulatory elements.

Construction of pKT-pelB.

Plasmid pK-pelB was further modified to include two regulatory elements upstream of the pelB leader, to create pKT-pelB (4.8 kb). These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron, ATG TAT CGA TTA AAT AAG GAG GAA TAA (SEQ ID NO.28), coding for 8 amino acid residues. Insertion of the two regulatory elements was achieved by digestion of pK2D with *Spe*I and *Sac*I, followed by ligation of the vector to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the pelB leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 71-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp,

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respectively) and Pair B (oligos J5B + J6B, 71 bp and 76 bp, respectively) having the following sequence:

J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA
AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA
ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5B

5' - ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTC
GCTGCCCAACCAGCCATGGCCGAGCT - 3' [SEQ ID NO 11]

J6B

5' - CGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCGGCTG
CCGTAGGCAATAGGTATTTCATTATTTATTC - 3' [SEQ ID NO 12]

Construction of pKT-STII.

Plasmid pK-STII was further modified to include the same two regulatory elements as in pKT-pelB, thus creating pKT-STII (4.8 kb). pK2D was digested with *Spe*I and *Dra*III, and the vector was ligated to synthetic oligonucleotides coding for the g10 sequence, the ribosome binding site, a mini-cistron and the STII leader, in the 5' to 3' direction. The oligonucleotides were synthesized as two complementary sets of 73-89 mers that could be ligated via complementary 9 bp overhangs between the pairs of oligonucleotides. The two complementary pairs of oligonucleotides were designated Pair A (oligos J5A + J6A, 89 bp and 76 bp, respectively) and Pair B (oligos J5C + J6C, 73 bp and 79 bp, respectively) having the following sequence:

J5A

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTAA
AGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAATA - 3' [SEQ ID NO 9]

J6A

5' - CTCCTTATTTAATCGATACATTAATATATACCTCTTTAATTTTTA
ATAATAAAGTTAATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 10]

J5C

5' - ATGAAAAAGAATATAGCATTCCTACTAGCTTCAATGTTCGTCTT
CTCTATTGCAACTAACGCGTACGCACATT - 3' [SEQ ID NO 13]

J6C

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5' - GTGCGTACGCGTTAGTTGCAATAGAGAAGACGAACATTGAAGC
TAGTAGGAATGCTATATTCTTTTTCATTATTTATTC - 3' [SEQ ID NO 14]

EXAMPLE 4

Construction of pK-pelB-Vβ5.3.

The next step in the expression of the T cell antigen receptor Vβ5.3 protein was the incorporation of the Vβ5.3 sequence into the plasmids containing the signal sequences as described in Example 2 *supra*.

The complete nucleotide sequence of Vβ5.3 was generated as follows: the DNA sequence of Vβ5.3 clone 12A1 was originally published by Leiden et al. (1986, *Proc. Natl. Acad. Sci. USA*, 83:4456-4460) and later partially corrected (Leiden et al., 1986, *Molec. Cell Biol.*, 6:3207-3214). This clone 12A1 was missing nucleotides coding for the N-terminal four amino acid residues; and in addition, the deduced N-terminal amino acid residues of clone 12A1 were incorrect. The N-terminal amino acid sequence of Vβ5.3 (HPB-ALL) was published by Jones et al., 1985, *Science*, 227:311-314) without specifying the N-terminal first amino acid residue, which was later determined to be Glycine (Jeff Leiden, unpublished). Using the above sequence information, the complete Vβ5.3 was reconstructed by recombinant DNA techniques. The resulting plasmid (pBB-Vβ5.3) was the source of the Vβ5.3 sequence for the following examples. The full length nucleotide sequence coding for Vβ5.3 and the predicted amino acid sequence have been published more recently by Plaza et al. (1991, *J. Immunol.*, 147:4360-4365). (See, also, Figure 1; SEQ ID NOS. 25, 26 and 27 herein.)

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TAATTAGCCATGGCCGGCGTAACCCAATCTCCG - 3' [SEQ ID NO 15]

The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 16]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *EcoR*V site of pBluescript II KS+ to yield plasmid pBL-pelB- Vβ5.3. The Vβ5.3 insert was excised by digestion with *Nco*I and *Pst*I, purified from agarose, and ligated into the *Nco*I and *Pst*I sites of pK-pelB, yielding plasmid pK-pelB- Vβ5.3 (5 kb).

Construction of pK-STII-Vβ5.3.

Vβ5.3 was PCR-synthesized using pBB- Vβ5.3 (6.2 kb) as template. The 5' "sense" primer contained an *Mlu*I restriction enzyme site, underlined:

5' - GAAATTAACGCGTACGCAGGCGTAACCCAATCTC - 3' [SEQ ID NO 17]

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The 3' "antisense" primer was complementary to a region downstream of a *Pst*I site in the vector:

5' - CCAGTGCCAAGCTTGCATGCC - 3' [SEQ ID NO 18]

The resulting Vβ5.3 fragment was blunt-ended using the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated *EcoR*V site of pBluescript II KS+ to yield plasmid pBL-STII- Vβ5.3. The Vβ5.3 insert was excised by digestion with *Mlu*I and *Pst*I, purified from agarose, and ligated into the *Mlu*I and *Pst*I sites of pK-STII, yielding plasmid pK-STII- Vβ5.3 (5 kb).

Results

Vβ5.3 fused to the pelB or the STII leaders was expressed in *E. coli* JM109 (TABLE 1). Induction was for 2 hr. and Vβ5.3 yields were measured by EIA.

TABLE 1

EXPRESSION AND COMPARTMENTATION OF Vβ5.3

	pK-pelB-Vß5.3		pK-STII-Vß5.3	
Compartment	0 mM IPTG	1 mM IPTG	0 mM IPTG	1 mM IPTG
Supernatant	0 µg/ml	1.3 µg/ml	0.5 µg/ml	2.2 µg/ml
Periplasm	0.9	4.6	N.D.	5.5
Cytosol	9.9	Off scale [1:40]	2.0	Off scale [1:40]

Both leaders were functional, although most of the expressed protein was localized intracellularly.

EXAMPLE 5

This Example details the plasmids containing the leader sequence and the two regulatory elements (g10 and mini-cistron) as well as the Vβ5.3 sequence.

Construction of pKT-pelB-Vß5.3

Plasmid pKT-pelB-Vβ5.3 was constructed by excision of the Vβ5.3 insert from pK-pelB-Vβ5.3 using *Nco*I and *Pst*I, and ligation into the *Nco*I and *Pst*I sites of pKT-pelB.

Construction of pKT-STII-Vß5.3

Plasmid pKT-STII-Vβ5.3 was similarly constructed by excision of the Vβ5.3 insert from pK-STII-Vβ5.3 using *Mlu*I and *Pst*I, and ligation into the *Mlu*I and *Pst*I sites of pKT-STII.

All subsequent plasmid constructs utilized the STII signal sequence. Therefore, where the signal sequence is not specified, it is understood that the STII sequence was utilized.

Results

pKT-Vß5.3

Plasmid pK-STII-Vβ5.3 (Example 4) was modified to include two regulatory elements upstream of the STII leader, to create pKT-Vβ5.3. These elements are the translational enhancer from the bacteriophage T7 gene 10 (g10), and a mini-cistron coding for 8 amino acid residues. Both elements have been demonstrated to enhance translational efficiency of several genes (Schoner et al., 1984, *Proc. Natl. Acad. Sci. USA*, 81:5403-5407, Schoner et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:8506-8510; Olins et al., 1988, *Gene*, 73:227-235; Olins and Rangwala, 1989, *J. Biol. Chem.*, 264:16973-16976).

pKT-Vβ5.3 provided up to 8-fold higher protein yields than pK-STII-Vβ5.3 (Example 4) in *E. coli* JM109 under conditions of IPTG induction. The EIA values reported here are approximations, due to the lack of a pure Vβ5.3 standard at this time. pKT-Vβ5.3 was shown to be stable in *E. coli* JM109.

EXAMPLE 6

Construction of pKTB-Vß5.3.

A further modification to pKT-Vβ5.3 (Example 5) for secreted expression involved elimination of the IPTG requirement for induction. The existence of a *lacI*ts gene coding for temperature-sensitive *lac* repressor (Bukrinsky et al., 1988, *Gene*, 70:415-417) makes it possible to control gene expression from the *trc* promoter by elevating the temperature from 30°C to 42°C. This would obviate the need to use IPTG or bacterial strains harboring the *lacI* or *lacI*^{q} genes.

The *lacI*ts gene was excised from pUCts by *EcoR*I digestion. The 1.7 kb fragment containing the entire *lacI*ts-coding region and its associated regulatory elements, flanked by vector sequence, was blunt-ended with Klenow, and ligated into the dephosphorylated ScaI site, located downstream of the rrnB operon in pKT-Vβ5.3, yielding plasmid pKTB-Vβ5.3 (6.8 kb). The orientation of the *lacI*ts gene was determined by restriction mapping.

Results

The *lacI*ts gene was inserted in either orientation downstream of the *rrnB* transcription terminators in pKT-Vβ5.3 to create plasmid pKTB-Vβ5.3 (Fig. 2). This was expressed in *E. coli* LJ24 (*lacI* deletion) and the protein was analyzed by Coomassie Blue staining on acrylamide gels (Laemmli, (1970) *supra*) and by Western blot (Towbin et al.,

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(1979) *supra*). One orientation of the *lacI*ts gene (transcription direction toward the Amp^R region) clearly resulted in higher Vβ5.3 yield than the reverse orientation.

The putative Vβ5.3 band obtained from extracts of total cellular pellets was electrotransferred to Immobilon-P membranes, and visualized by Coomassie Blue staining. The band was excised and subjected to NH_2-terminal amino acid sequencing (Matsudaira, 1987, *J. Biol. Chem.*, 262:10035-10038) to yield a total of 10 amino acid residues. These matched precisely the sequence of the N-terminal 10 amino acid residues of the processed Vβ5.3, i.e. no leader sequence was detected.

This plasmid was also expressed by thermal induction in three different bacterial strains, each of which contains increasing levels of endogenous *lac* repressor, i.e., LJ24<DH5α<JM109. Supernatants from induced cultures were assayed for Vβ5.3 expression by EIA (Table2)

TABLE 2

pKTB-Vß5.3 EXPRESSION IN DIFFERENT BACTERIAL STRAINS

Cells were induced for 2 hr., and Vβ5.3 yields (μg/ml) were measured in culture supernatants.

Plasmid	Regulation	*E. coli* Host	Induction	Uninduced	Induced
pKTB-Vβ5.3	*lacI*ts	JM109 (lacI^q)	42°C	N.D.	0.5
pKTB-Vβ5.3	*lacI*ts	DH5α (lacI)	42°C	N.D.	5.0
pKTB-Vβ5.3	*lacI*ts	LJ24 (Δ*lacI*)	42°C	N.D.	5.2
pKT-Vβ5.3	*lacI*^{q}	JM109	2mM IPTG	0	0.9
pKT	*lacI*^{q}	JM109	42°C	N.D.	0

The data agree with Western blot observations and predicted results, i.e., the highest expression level was achieved in LJ24 (Δ*lacI*) and the lowest in JM109 (*lacI*^{q}). The comparatively low amounts of Vβ5.3 measured in the culture supernatants follow from the fact that *E. coli* does not ordinarily secrete proteins into the medium. As shown in Table 1, the bulk of the expressed Vβ5.3 is retained in the periplasm.

DNA sequencing of the *lacI*ts gene.

The entire coding sequence of the temperature-inducible *lacI*ts gene plus the upstream 80 nucleotides including the promoter were sequenced and compared to the published sequence of the IPTG-inducible *lacI* gene (Farabaugh, 1978, *Nature*, 274:765-769). The promoter region was identical to that of the *lacI*, and not *lacI*^{q}, gene (Calos M. P., 1978, *Nature*, 274:762-765). The *lacI*ts gene sequence showed an

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open reading frame of 1080 nucleotides encoding 360 amino acids, and differed from the *lacI* gene at nucleotide #559 (with reference to first nucleotide of the start codon), which changed from G to A, causing amino acid residue #187 to change from Glycine (GGC) to Serine (AGC). This change in a single amino acid residue imparts thermal sensitivity to the *lac* repressor protein.

In addition, the published sequence of the *lacI* gene (Farabaugh, (1978) *supra*) as well as the sequence appearing in Genebank (locus ECOLAC) contains an error at position #857: nucleotide #857 should be T instead of C, which would make residue #286 code for Leucine (TTA) instead of Serine (TCA). The C-T transition introduces a *Hpa*I site at the 3' end of the *lacI* gene that was shown previously by restriction mapping (Brosius, 1992, *Meth. Enzymol.*, 216:469-483). It should be noted that in Farabaugh, (1978) *supra*, the same codon (TCA) is erroneously reported as encoding Leucine instead of Serine.

EXAMPLE 7

Construction of pKTB.

Plasmid pKTB-Vβ5.3 (6.7 kb) was digested with *Sal*I and *Pst*I, and the 5.42 kb fragment was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pKT-STII (4.8 kb) was digested with *Sal*I and *Pst*I, and the 1.15 kb fragment was similarly purified, and ligated to the 5.42 kb fragment, yielding plasmid pKTB (6.56 kb).

EXAMPLE 8

Construction of pKB-Vβ5.3.

This plasmid is identical to pKTB-Vβ5.3 minus the two regulatory elements, i.e., the g10 translational enhancer and the mini-cistron. Plasmid pKTB (6.56 kb) was digested with *Sal*I and *Pst*I, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-Vβ5.3 (4.9 kb) was digested with *Sal*I and *Pst*I, and the 1.3-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB-Vβ5.3 (6.7 kb).

Results

pKB-Vβ5.3

As discussed above, the addition of two regulatory elements, i.e., the g10 gene segment and the mini-cistron, appeared to enhance Vβ5.3 yield under conditions of IPTG induction. We wished to remove these two elements from the pKTB-Vβ5.3 vector, and reexamine Vβ5.3 yield under conditions of thermal induction. A comparison of pKTB-Vβ5.3 and pKB-Vβ5.3 by acrylamide electrophoresis and Western blotting showed

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that Vβ5.3 yield was unaffected by the removal of these two elements. EIA measurements of whole cell urea extracts showed that pKB-Vβ5.3 produced more protein than pKTB-Vβ5.3 when expressed in *E. coli* LJ24.

TABLE 3

EXPRESSION OF Vβ5.3 IN DIFFERENT BACTERIAL STRAINS

Plasmid	LJ24	SG22094	SG21163	SG21173
pKTB-Vß5.3 + leader + g10/MC	90	80	20	30
pKB-Vß5.3 + leader - g10/MC	110	50	40	20
pKTBi-Vß5.3 - leader + g10/MC	6	100	100	100
pKBi-Vß5.3 - leader -g10/MC	10	170	130	390

Induction period: 2 hr at 42°C
Culture volume: 25 ml
Leader: STII
g10: T7 gene 10
MC: Mini-cistron
Samples: whole cell urea extracts
Vβ5.3 yields (mg/liter), based on units/ml approximations, as measured by EIA. 1 mg Vβ5.3 is approximately equivalent to 1 x 10^7 units.

Plasmid pKB-Vβ5.3 was demonstrated to be stable for fermentation.

EXAMPLE 9

Construction of pKB

Plasmid pKTB (6.56 kb) was digested with *SalI* and *PstI*, and the large fragment (5.4 kb) was purified from agarose using the Geneclean Kit (BIO 101). Plasmid pK-STII (4.75 kb) was digested with *SalI* and *PstI*, and the 1.1-kb fragment was similarly purified, and ligated to the 5.4-kb fragment, yielding plasmid pKB (6.5 kb).

EXAMPLE 10

Construction of pKTBi.

This plasmid is identical to pKTB minus the STII signal sequence. pKTB (6.56 kb) was digested with *SpeI* and *XmaI*, and ligated to a double-stranded synthetic oligonucleotide

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coding for the g10 translational enhancer, a ribosome binding site, and a mini-cistron containing a second ribosome binding site. Restriction sites for *Nco*I, *Dra*III, *Rsr*II and *Xma*I were designed in the 3' end. The two complementary oligonucleotides had the following sequences:

5' - CTAGTCCGGAATTGGGCATCGATTAACTTTATTATTAAAAATTA
AAGAGGTATATATTAATGTATCGATTAAATAAGGAGGAATAAACC
ATGGCACATTGTGCGGTCCGC - 3' [SEQ ID NO 19]

5' - CCGGGCGGACCGCACAATGTGCCATGGTTTATTCCTCCTTATTTA
ATCGATACATTAATATATACCTCTTTAATTTTTAATAATAAAGTTA
ATCGATGCCCAATTCCGGA - 3' [SEQ ID NO 20]

EXAMPLE 11

Construction of pKBi

This plasmid is identical to pKB minus the STII signal sequence. pKTB (6.56 kb) was digested with *Spe*I and *Dra*III, and ligated to a double-stranded synthetic oligonucleotide containing a ribosome binding site, and *Nco*I and *Dra*III sites designed in the 3' end. The two complementary oligonucleotides had the following sequences:

5' - CTAGTAAATTATATTTAAAGGAGGAATAAACCATGGCACATT - 3' [SEQ ID NO 21]

5' - GTGCCATGGTTTATTCCTCCTTTAAATATAATTTA - 3' [SEQ ID NO 22]

EXAMPLE 12

Construction of pKTBi-Vß5.3

This plasmid is identical to pKTB-Vß5.3 minus the STII signal sequence. pKTBi (6.4 kb) was digested with *Xma*I and *Nco*I and ligated to PCR-synthesized Vβ5.3 using pKTB-Vβ5.3 as template. The 5' "sense" primer contained an *Nco*I restriction enzyme site, underlined:

5' - TATAGTCCATGGGCGTAACC - 3' [SEQ ID NO 23]

The 3' "antisense" primer contained an *Xma*I site, underlined:

5' - AACTTCCCGGGTTATCATTAGCTGC - 3' [SEQ ID NO 24]

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Results

pKTBi-Vß5.3

In parallel with the above work, the STII leader was removed from the expression plasmid. Since most of the expressed Vβ5.3 is retained intracellularly (also see below), it was expected that scale-up of this construct might yield a heterogeneous population of Vβ5.3 molecules, i.e., plus and minus leader, which would complicate subsequent purification of the processed Vβ5.3. Therefore, the STII leader was removed to create pKTBi-Vβ5.3 for intracellular expression. The expressed Vβ5.3 is expected to contain an N-terminal methionine, although this amino acid might be removed by endogenous methionine aminopeptidases (Ben-Bassat et al., 1987, *J. Bacteriology*, 169:751-757; Baneyx and Georgiou, "Expression of Proteolytically Sensitive Polypeptides in *Escherichia coli*", in Stability of Protein Pharmaceuticals, Part A: Chemical and Physical Pathways of Protein Degradation (Tim J. Ahern and Mark C. Manning, eds.), Plenum Press, New York, pp. 69-108 (1992)), as observed for pKBi-Vβ5.3 (below).

pKTBi-Vβ5.3 was completed, but it failed to express significant levels of protein in *E. coli* LJ24 (see Table 3, *supra*). The entire Vβ5.3 insert was sequenced and determined to be unambiguously correct. Plasmid DNA isolated post-induction and analyzed by restriction digestion appeared to be unaltered. One possible explanation for the apparent failure of the LJ24 strain containing pKTBi-Vβ5.3 to express significant levels of Vβ5.3 protein is that the absence of a leader peptide destabilizes intracellular Vβ5.3 protein, and leads to its rapid proteolysis.

Therefore, pKTBi-Vβ5.3 was transformed into the protease-deficient strains SG22094 (lon$^-$, clp$^-$), and BL21 (lon$^-$, ompT$^-$) which gave decreasing yields in the order of SG22904>BL21. The effect of different induction temperatures on yield was also examined, and for both strains 42°C was optimal. In addition, there was no significant increase in production between 2 and 5 hr.

pKTBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon$^-$, htpr$^-$) and SG21173 (lon$^-$, htpr$^-$, clp$^-$). The results are summarized in Table 3 (*supra*).

It should be noted that strains LJ24, SG22094, SG21163 and SG21174 do not produce endogenous *lac* repressor because the *lacI* gene is deleted in all of these strains. Thus, the wild type *lac* repressor does not interfere with the thermal induction of the *trc* promoter. The foregoing examples show that Vβ5.3 constucts without a signal peptide can be readily expressed in protease deficient hosts, resulting in production of significant amounts of Vβ5.3 protein.

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EXAMPLE 13

Construction of pKBi-Vß5.3

This plasmid is identical to pKTBi-Vβ5.3 minus the g10 element and the mini-cistron (SEQ ID NO. 28). Plasmid pKBi (6.43 kb) was digested with *Nco*I and *Sal*I, and a 970 bp fragment was purified from agarose. pKTBi-Vβ5.3 (6.75 kb) was digested with *Nco*I and *Sal*I and the 5.68 kb fragment was similarly purified, and ligated to the 970 bp fragment, yielding plasmid pKBi-Vβ5.3 (6.64 kb).

Results

pKBi-Vß5.3

This leaderless Vβ5.3 construct minus the two regulatory elements was successfully expressed in protease-deficient strains; our observations are similar to those for pKTBi-Vβ5.3. pKBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon^-, $htpr^r$) and SG21173 (lon^-, $htpr^r$, clp^-). The results are summarized in Table 3. pKBi-Vβ5.3 consistently produced more protein than pKTBi-Vβ5.3 in all strains tested (Table 3). N-terminal sequencing of the expressed protein showed that the first 17 amino acid residues matched the expected Vβ5.3 sequence, and the terminal methionine residue was absent. The plasmid was also demonstrated to be stable.

The present invention is not meant to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modification are intended to fall within the scope of the appended claims. Various publications are incorporated by reference in their entirety.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:T Cell Sciences, Inc

(ii) TITLE OF INVENTION:T Cell Antigen Receptor V Region Proteins and Methods of Preparation Thereof

(iii) NUMBER OF SEQUENCES:29

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY:Needham
(D) STATE:Massachusetts
(E) COUNTRY:United States of America
(F) ZIP:02194-2725

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE:Diskette, 3.50 inch, 1.44Mb storage
(B) COMPUTER:IBM PC Compatible
(C) OPERATING SYSTEM:MS-DOS
(D) SOFTWARE:WordPerfect 6.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER:08/181,492
(B) FILING DATE:13-January-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER:TCS-203-PCT(94,664-A)

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE:617-345-9100
(B) TELEFAX:617-345-9111

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:34 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

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(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TATAATGACT AGTCGCTGCA GCCAACCGCG GCTG 34

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:23 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTCCTAGAGT ACTGAGCGGA TAC 23

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:91 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

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(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGCTCGAGCC TAGGCTGCAG CCCGGGGCGC GCGCGGCCGC AGGCCTTTAA 50

TTAAGAGCTC CGGACCGCAC AATGTGGGCG CGCCCTTAAG A 91

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTAGTCTTAA GGGCGCGCCC ACATTGTGCG GTCCGGAGCT CTTAATTAAA 50

GGCCTGCGGC CGCGCGCGCC CCGGGCTGCA GCCTAGGCTC GAGCCGC 97

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

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(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAATA CCTATTGCCT 50

ACGGCAGCCG CTGGATTGTT ATTACTCGCT GCCCAACCAG CCATGGCCGA 100

GCT 103

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:95 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

GGCAATAGGT ATTTCATTAT GACTGTCTCC TTGAAATAGA ATTTA 95

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:105 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

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(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTAGTAAATT CTATTTCAAG GAGACAGTCA TAATGAAAAA GAATATAGCA 50

TTCCTACTAG CTTCAATGTT CGTCTTCTCT ATTGCAACTA ACGCGTACGC 100

ACATT 105

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:98 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATGACTGTC TCCTTGAAAT AGAATTTA 98

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:89 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAATA 89

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:76 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTCCTTATTT AATCGATACA TTAATATATA CCTCTTTAAT TTTTAATAAT 50

AAAGTTAATC GATGCCCAAT TCCGGA 76

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:71 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGAAATACC TATTGCCTAC GGCAGCCGCT GGATTGTTAT TACTCGCTGC 50

CCAACCAGCC ATGGCCGAGC T 71

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CGGCCATGGC TGGTTGGGCA GCGAGTAATA ACAATCCAGC GGCTGCCGTA 50

GGCAATAGGT ATTTCATTAT TTATTC 76

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATGAAAAAGA ATATAGCATT CCTACTAGCT TCAATGTTCG TCTTCTCTAT 50

TGCAACTAAC GCGTACGCAC ATT 73

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:79 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GTGCGTACGC GTTAGTTGCA ATAGAGAAGA CGAACATTGA AGCTAGTAGG 50

AATGCTATAT TCTTTTTCAT TATTTATTC 79

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:33 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

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(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAATTAGCCA TGGCCGGCGT AACCCAATCT CCG 33

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:34 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:
(ix) FEATURE:

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(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GAAATTAACG CGTACGCAGG CGTAACCCAA TCTC 34

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:21 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCAGTGCCAA GCTTGCATGC C 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

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CTAGTCCGGA ATTGGGCATC GATTAACTTT ATTATTAAAA ATTAAAGAGG 50

TATATATTAA TGTATCGATT AAATAAGGAG GAATAAACCA TGGCACATTG 100

TGCGGTCCGC 110

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:110 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CCGGGCGGAC CGCACAATGT GCCATGGTTT ATTCCTCCTT ATTTAATCGA 50

TACATTAATA TATACCTCTT TAATTTTTAA TAATAAAGTT AATCGATGCC 100

CAATTCCGGA 110

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:42 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:

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(B)LOCATION:

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTAGTAAATT ATATTTAAAG GAGGAATAAA CCATGGCACA TT 42

(2)INFORMATION FOR SEQ ID NO: 22:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:35 base pairs
(B)TYPE:nucleic acid
(C)STRANDEDNESS:single
(D)TOPOLOGY:linear

(ii)MOLECULE TYPE:other nucleic acid
(A)DESCRIPTION:synthetic oligonucleotide

(iii)HYPOTHETICAL:

(iv)ANTI-SENSE:

(ix)FEATURE:
(A)NAME:
(B)LOCATION:

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTGCCATGGT TTATTCCTCC TTTAAATATA ATTTA 35

(2)INFORMATION FOR SEQ ID NO: 23:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:20 base pairs
(B)TYPE:nucleic acid
(C)STRANDEDNESS:single
(D)TOPOLOGY:linear

(ii)MOLECULE TYPE:other nucleic acid
(A)DESCRIPTION:synthetic oligonucleotide

(iii)HYPOTHETICAL:

(iv)ANTI-SENSE:

(ix)FEATURE:
(A)NAME:
(B)LOCATION:

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 23:

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TATAGTCCAT GGGCGTAACC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:25 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:other nucleic acid
(A) DESCRIPTION:synthetic oligonucleotide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AACTTCCCGG GTTATCATTA GCTGC 25

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA 39

GGA CAG CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG 78

CAC AAG AGT GTG TCC TGG TAC CAA CAG GTC CTG GGT CAG 117

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GGG CCC CAG TTT ATC TTT CAG TAT TAT GAG AAA GAA GAG 156

AGA GGA AGA GGA AAC TTC CCT GAT CGA TTC TCA GCT CGC 195

CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT GTG AAC GCC 234

TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC AGC 273

AGC 276

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:276 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CCG CAT TGG GTT AGA GGC TGA GTG GAC TAG TTT TGC TCT 39

CCT GTC GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC 78

GTG TTC TCA CAC AGG ACC ATG GTT GTC CAG GAC CCA GTC 117

CCC GGG GTC AAA TAG AAA GTC ATA ATA CTC TTT CTT CTC 156

TCT CCT TCT CCT TTG AAG GGA CTA GCT AAG AGT CGA GCG 195

GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA CAC TTG CGG 234

AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG TCG 273

TCG 276

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:92 amino acids

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(B) TYPE:amino acid
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:protein

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln
 1               5                  10                  15
His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val
            20                  25                  30
Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe
            35                  40                  45
Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp
            50                  55                  60
Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn
            65                  70                  75
Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala
            80                  85                  90
Ser Ser
    92
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:27 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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ATG TAT CGA TTA AAT AAG GAG GAA TAA 27

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(ix) FEATURE:
(A) NAME:
(B) LOCATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Met Tyr Arg Leu Asn Lys Glu Glu
1 5 8

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We claim:

1. A method for producing a recombinant full length T cell antigen receptor V region protein free of extraneous protein sequences which comprises:

a) transforming a competent bacterial host cell with a recombinant expression vector comprising a region coding for a full length T cell antigen receptor V region protein, and an inducible bacterial promoter;

b) inducing the inducible bacterial promoter in the transformed bacterial host cell of step a, and;

c) recovering the recombinant full length T cell antigen receptor V region protein free of extraneous protein sequences.

2. The method of claim 1 wherein the inducible bacterial promoter is selected from the group consisting of chemically regulated promoters and thermally regulated promoters.

3 The method of claim 2 wherein the expression vector further comprises a regulatory gene sequence selected from the group consisting of the *lacI* gene, the *lacI*^{q} gene, and the *lacI*ts gene.

4. The method of claim 3 wherein the *lacI*ts gene is in the same orientation as the coding region for T cell antigen receptor V region protein.

5. The method of claim 1 wherein the bacterial promotor is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

6. The method of claim 5 wherein the bacterial host strain is a protease-deficient strain.

7. The method of claim 5 wherein the recombinant expression vector further comprises a leader sequence.

8. The method of claim 7 wherein the leader sequence is selected from the group consisting of heat stable enterotoxin STII leader from *Escherichia coli* and a pelB leader from *Erwinia carotovora*.

9. The method of claim 8 wherein the recombinant expression vector further comprises a leader sequence derived from the STII leader sequence of *E. coli* and the competent bacterial host cell is a

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cell which does not harbor the *lacI* or *lacI*^{q} gene.

10. The method of claim 9 wherein the bacterial host strain is a protease deficient strain.

11. The method according to claim 10, wherein the protease deficient strain is selected from the group consisting of SG21163 and SG21173.

12. The method according to claim 1, wherein the expression vector further comprises the g10 and the mini-cistron sequence (SEQ ID NO. 28) regulatory elements.

13. The method of claim 1 wherein the recombinant expression vector further comprises;

a) a *trc* promotor as the inducible bacterial promoter;

b) a ribosomal binding site;

c) a transcription terminator;

d) a *lacI*ts regulatory gene sequence;

e) a pBR322 origin of replication, and;

f) a tetracycline resistance gene.

14. The method of claim 13 wherein the recombinant expression vector is selected from the group consisting of pKTBi-Vß5.3, and pKBi-Vß5.3.

15. The method of claim 13 wherein the recombinant expression vector further comprises a leader sequence.

16. The method of claim 15 wherein the recombinant expression vector is selected from the group consisting of pK-STII-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, and pKB-Vß5.3.

17. An expression vector comprising a inducible bacterial promoter under the control of a *lacI*ts gene, wherein said *lacI*ts gene controls the expression of a region encoding a full length T cell antigen receptor V region protein which full length T cell antigen receptor V region protein is expressed free of extraneous protein sequences.

18. The expression vector of claim 17 wherein the inducible bacterial promoter is selected from the group consisting of a *lac* promoter, a *tac* promoter, and a *trc* promoter.

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19. The expression vector of claim 18 which further comprises a leader sequence.

20. The expression vector of claim 17 which comprises in a 5' to 3' direction;

a) a *trc* promotor;

b) a ribosomal binding site;

c) a region encoding a full length T cell antigen receptor V region protein;

d) a transcription terminator;

e) a *lacI*ts regulatory gene sequence;

f) a pBR322 origin of replication, and;

g) a tetracycline resistance gene.

21. The expression vector of claim 17 wherein said vector is selected from the group consisting of pK-STII-Vß5.3, pKT-Vß5.3, pKTB-Vß5.3, pKTBi-Vß5.3, and pKBi-Vß5.3.

22. A bacterial cell transformed with the expression vector according to claim 17.

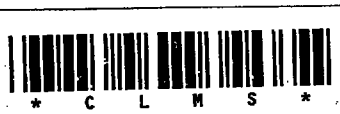
23. The transformed bacterial cell according to claim 22 which produces at least 300 milligrams of full length recombinant T cell antigen receptor Vβ protein per liter of cell culture.

24. An isolated full length T cell antigen receptor V region protein produced by the method of claim 13.

25. A pharmaceutical composition which comprises a full length T cell antigen receptor V region protein.

26. The pharmaceutical composition of claim 25 wherein the T cell antigen receptor V region protein is the Vß5.3 protein and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.

27. A method for treating an immune related disorder comprising administering to a subject in need of such treatment a pharmaceutical composition comprising a full length T cell antigen receptor V region protein.



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28. The method according to claim 27 wherein the immune related disorder is multiple sclerosis.

29. The method of claim 28 wherein the full length T cell antigen receptor V region protein is the Vß5.3 and has the formula: amino acid 1 to 92 of amino acid Sequence ID No. 27.

30. An isolated full length T cell antigen receptor Vβ5.3 protein having the formula: amino acids 1-92 of amino acid Sequence ID No. 27.

31. The use of a substantially pure T cell antigen receptor V region protein in the manufacture of a medicament for treatment of an immune-related disease.

32. An isolated full length T cell antigen receptor V region protein according to claim 24 or claim 30 for use in therapy.

33. An isolated full length T cell antigen receptor V region protein according to claim 24 or claim 30 for use in the diagnosis of an immune-related disease.

34. A method of diagnosing an immune disorder comprising using a recombinant full length T cell antigen receptor V region protein to detect in a biological sample from a patient the presence of a particular subpopulation of T cells associated with an immune disorder or the presence of autoantibodies recognizing the particular V region protein.

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T CELL ANTIGEN RECEPTOR V REGION PROTEINS AND METHODS OF PREPARATION THEREOF

ABSTRACT

The present invention relates to the production of full length T cell antigen receptor V region proteins. The invention provides for methods of expressing the proteins as well as expression vectors for enhanced production of full length T cell antigen receptor V region proteins. The invention further provides for the Vβ5.3 region protein. The proteins of the invention have uses in the diagnosis and therapy of immune-related disorders.

FIGURE 2.

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Figure 1

```
  1  GGC GTA ACC CAA TCT CCG ACT CAC CTG ATC AAA ACG AGA GGA CAG
     CCG CAT TGG GTT AGA GGC TGA CTG GAC TAG TTT TGC TCT CCT GTC
  1  Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln

 46  CAC GTG ACT CTG AGA TGC TCT CCT ATC TCT GGG CAC AAG AGT GTG
     GTG CAC TGA GAC TCT ACG AGA GGA TAG AGA CCC GTG TTC TCA CAC
 16  His Val Thr Leu Arg Cys Ser Pro Ile Ser Gly His Lys Ser Val

 91  TCC TGG TAC CAA CAG GTC CTG GGT CAG GGG CCC CAG TTT ATC TTT
     AGG ACC ATG GTT GTC CAG GAC CCA GTC CCC GGG GTC AAA TAG AAA
 31  Ser Trp Tyr Gln Gln Val Leu Gly Gln Gly Pro Gln Phe Ile Phe

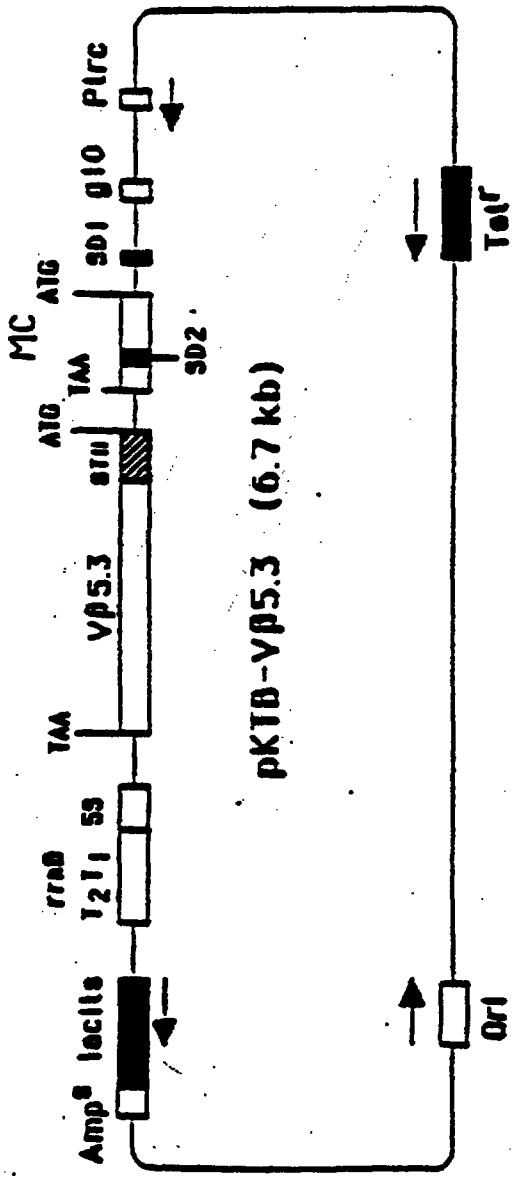
136  CAG TAT TAT GAG AAA GAA GAG AGA GGA AGA GGA AAC TTC CCT GAT
     GTC ATA ATA CTC TTT CTT CTC TCT CCT TCT CCT TTG AAG GGA CTA
 46  Gln Tyr Tyr Glu Lys Glu Glu Arg Gly Arg Gly Asn Phe Pro Asp

181  CGA TTC TCA GCT CGC CAG TTC CCT AAC TAT AGC TCT GAG CTG AAT
     GCT AAG AGT CGA GCG GTC AAG GGA TTG ATA TCG AGA CTC GAC TTA
 61  Arg Phe Ser Ala Arg Gln Phe Pro Asn Tyr Ser Ser Glu Leu Asn

226  GTG AAC GCC TTG TTG CTG GGG GAC TCG GCC CTG TAT CTC TGT GCC
     CAC TTG CGG AAC AAC GAC CCC CTG AGC CGG GAC ATA GAG ACA CGG
 76  Val Asn Ala Leu Leu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala

271  AGC AGC     [SEQ ID NO. 25]
     TCG TCG     [SEQ ID NO. 26]
 91  Ser Ser     [SEQ ID NO. 27]
```

Figure 2



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Others have produced chimeric molecules by shuffling the variable and constant domains of the murine TCR with the constant region of an immunoglobulin κ light chain. The investigators were able to achieve small amounts of a secreted soluble form of a heterodimeric αß TCR (Gregoire, C., et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8077-8081). Gregoire et al. show a murine chimera consisting of the Cα and Vα genes of the KB5-C2 joined to the C region of the κ light chain of the S105 monoclonal antibody. Also shown is a VßCßCκ chimera. Both are transfected into a mammalian B cell myeloma that does not express native immunoglobulin heavy or light chains. (See also, Weber, S., et al., 1992, *Nature*, 356:793-795).

Novotny et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8646-8650, demonstrate a method for producing soluble TCRs with antigen-combining properties. A synthetic oligo linker was cloned onto the C terminus of the Vß and the N terminus of the Vα region resulting in a single-chain VßVα. The resultant expression vector encodes the pelB leader followed immediately by in frame Vß, the linker, and the Vα segments. The expression of this construct is under the control of the lacZ promoter and is therefore IPTG-inducible.

Recently, one group has shown the secretion of a small amount of a T cell receptor V region fused to a carboxyl-terminal polyhistidine "tail". The plasmids contain the pelB leader and are under the control of a chemically inducible promoter (Ward, E.S., 1991, *Scand. J. Immunol.*, 34:215-220). The work fails, however, to describe how to obtain a T cell antigen receptor V region protein in the absence of a fusion partner, or how to obtain commercially useful amounts of the receptor V region.

There is a great need, therefore, for agents and pharmaceutical compositions which have the properties of specificity for the targeted autoimmune response, predictability in their selection, and convenience and reproducibility of preparation.

SUMMARY OF THE INVENTION

The present invention addresses the above-mentioned problems by providing for the first time the successful production of a universal agent for treating a given T cell-mediated disease in which T cell subsets are implicated. In particular, the invention provides useful quantities of full length TCR V region proteins that may be naturally processed, providing for multiple active fragments upon *in vivo* processing, and presented by antigen-presenting cells of each individual, thereby eliminating the need for "tailor-made" therapeutic agents. Also provided is a class of recombinant expression vectors for the production of TCR V region proteins in bacteria. The recombinant TCR V region proteins of the instant invention are useful in the treatment, prevention or suppression of an immune-related disorder. In another embodiment, the TCR molecules can be combined in an admixture with a pharmaceutically acceptable carrier for use as a therapeutic composition. In another embodiment, the claimed

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(1979) *supra*). One orientation of the *lacI*ts gene (transcription direction toward the Amp^R region) clearly resulted in higher Vβ5.3 yield than the reverse orientation.

The putative Vβ5.3 band obtained from extracts of total cellular pellets was electrotransferred to Immobilon-P membranes, and visualized by Coomassie Blue staining. The band was excised and subjected to NH_2-terminal amino acid sequencing (Matsudaira, 1987, *J. Biol. Chem.*, 262:10035-10038) to yield a total of 10 amino acid residues. These matched precisely the sequence of the N-terminal 10 amino acid residues of the processed Vβ5.3, i.e. no leader sequence was detected.

This plasmid was also expressed by thermal induction in three different bacterial strains, each of which contains increasing levels of endogenous *lac* repressor, i.e., LJ24<DH5α<JM109. Supernatants from induced cultures were assayed for Vβ5.3 expression by EIA (Table2)

TABLE 2

pKTB-Vß5.3 EXPRESSION IN DIFFERENT BACTERIAL STRAINS

Cells were induced for 2 hr., and Vβ5.3 yields (μg/ml) were measured in culture supernatants.

Plasmid	Regulation	*E. coli* Host	Induction	Uninduced	Induced
pKTB-Vβ5.3	*lacI*ts	JM109 (lacI^q)	42°C	N.D.	0.5
pKTB-Vβ5.3	*lacI*ts	DH5α (lacI)	42°C	N.D.	5.0
pKTB-Vβ5.3	*lacI*ts	LJ24 (Δ*lacI*)	42°C	N.D.	5.2
pKT-Vβ5.3	*lacI*^{R}	JM109	2mM IPTG	0	0.9
pKT	*lacI*^{R}	JM109	42°C	N.D.	0

The data agree with Western blot observations and predicted results, i.e., the highest expression level was achieved in LJ24 (Δ*lacI*) and the lowest in JM109 (*lacI*^{R}). The comparatively low amounts of Vβ5.3 measured in the culture supernatants follow from the fact that *E. coli* does not ordinarily secrete proteins into the medium. As shown in Table 1, the bulk of the expressed Vβ5.3 is retained in the cytoplasm.

DNA sequencing of the *lacI*ts gene.

The entire coding sequence of the temperature-inducible *lacI*ts gene plus the upstream 80 nucleotides including the promoter were sequenced and compared to the published sequence of the IPTG-inducible *lacI* gene (Farabaugh, 1978, *Nature*, 274:765-769). The promoter region was identical to that of the *lacI*, and not *lacI*^{R}, gene (Calos M. P., 1978, *Nature*, 274:762-765). The *lacI*ts gene sequence showed an

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Results

pKTBi-Vβ5.3

In parallel with the above work, the STII leader was removed from the expression plasmid. Since most of the expressed Vβ5.3 is retained intracellularly (also see below), it was expected that scale-up of this construct might yield a heterogeneous population of Vβ5.3 molecules, i.e., plus and minus leader, which would complicate subsequent purification of the processed Vβ5.3. Therefore, the STII leader was removed to create pKTBi-Vβ5.3 for intracellular expression. The expressed Vβ5.3 is expected to contain an N-terminal methionine, although this amino acid might be removed by endogenous methionine aminopeptidases (Ben-Bassat et al., 1987, *J. Bacteriology*, 169:751-757; Baneyx and Georgiou, "Expression of Proteolytically Sensitive Polypeptides in *Escherichia coli*", in Stability of Protein Pharmaceuticals, Part A: Chemical and Physical Pathways of Protein Degradation (Tim J. Ahern and Mark C. Manning, eds.), Plenum Press, New York, pp. 69-108 (1992)), as observed for pKBi-Vβ5.3 (below).

pKTBi-Vβ5.3 was completed, but it failed to express significant levels of protein in *E. coli* LJ24 (see Table 3, *supra*). The entire Vβ5.3 insert was sequenced and determined to be unambiguously correct. Plasmid DNA isolated post-induction and analyzed by restriction digestion appeared to be unaltered. One possible explanation for the apparent failure of the LJ24 strain containing pKTBi-Vβ5.3 to express significant levels of Vβ5.3 protein is that the absence of a leader peptide destabilizes intracellular Vβ5.3 protein, and leads to its rapid proteolysis.

Therefore, pKTBi-Vβ5.3 was transformed into the protease-deficient strains SG22094 (lon^-, clp^-), and BL21 (lon^-, $ompT^-$) which gave decreasing yields in the order of SG22904>BL21. The effect of different induction temperatures on yield was also examined, and for both strains 42°C was optimal. In addition, there was no significant increase in production between 2 and 5 hr.

pKTBi-Vβ5.3 was expressed in two additional protease-deficient strains: SG21163 (lon^-, $htpr^-$) and SG21173 (lon^-, $htpr^-$, clp^-). The results are summarized in Table 3 (*supra*).

It should be noted that strains LJ24, SG22094, SG21163 and SG21173 do not produce endogenous *lac* repressor because the *lacI* gene is deleted in all of these strains. Thus, the wild type *lac* repressor does not interfere with the thermal induction of the *trc* promoter. The foregoing examples show that Vβ5.3 constucts without a signal peptide can be readily expressed in protease deficient hosts, resulting in production of significant amounts of Vβ5.3 protein.