HUMAN B-CELL ANTIGENS; RELATED REAGENTS

FIELD OF THE INVENTION

The present invention relates to various biological reagents which are useful in modulating a human immune response. More particularly, it is directed towards compositions and methods useful, e.g., in B and T cell interaction.

BACKGROUND OF THE INVENTION

Leukemias, lymphomas, carcinomas, and other malignancies are described in, e.g., Wilson, et al. (eds.) Harrison's Principles of Internal Medicine, McGraw-Hill, New York, pp. 1599-1612). As one example of such diseases, malignant lymphomas are neoplastic transformed cells that reside predominantly in lymphoid tissues (see, e.g., Nadler, L.M. in Harrison's). Ninety percent of non-Hodgkins lymphomas, of which approximately 30,000 new cases occur each year in the U.S., are B cell lymphomas. Less than 25% of these cases are cured. Another example is leukemia, of which approximately 30,000 new cases occur annually in the U.S. Thus, a need exists for a more effective treatment for B and T cell lymphomas, leukemias, carcinomas, and other malignancies.

Growth of normal resting B cells (also referred to as "B lymphocytes") involves two distinct steps. First, the resting cells are activated to pass from the G_0 to G_1 phase of the cell cycle. See, e.g., Alberts, et al. (eds. 1989) Molecular Biology of the Cell Garland Publ., NY; and Darnell, et al. (1990) Molecular Cell Biology Freeman, NY. Next, the activated cells are induced to proliferate. See, e.g., Paul, ed. (1989) Fundamental Immunology, 2nd ed., Raven Press, NY; and the third edition. Several factors have been identified that induce growth of B cells, including interleukin-1 (IL-1), IL-2, IL-4, IL-10, and IL-13. In addition, antibodies against certain B cell surface molecules have been demonstrated to promote B cell proliferation. T cells

(also referred to as "T lymphocytes") are also induced to proliferate by certain factors, which include phytohemagglutinin, anti-T cell receptor monoclonal antibodies, anti-CD3 monoclonal antibodies, and other agents.

Numerous studies implicate the CD40 molecule as a growth factor receptor and an important regulator of human B cell proliferation and development. B lymphocytes are activated by the interaction of CD40 molecules on the surface of the B lymphocytes with a ligand that is transiently expressed on activated helper T cells.

The CD40 is a membrane-associated glycoprotein expressed on normal B lymphocytes and B cell malignancies, interdigitating cells, follicular dendritic cells, thymic epithelial cells, and some carcinomas. Human CD40 was first identified in 1985 as the epitope of a monoclonal antibody that is expressed almost exclusively on B lymphocytes, and therefore is a useful marker for B cells. The human CD40 antigen is a 45-50 Kd glycoprotein.

Anti-CD40 antibodies provide a potent co-stimulatory signal for B cell proliferation induced by either phorbol myristic acetate (PMA), anti-CD20 antibodies, or anti-immunoglobulin antibodies. The addition of anti-human CD40 antibodies plus IL-4 to activated B cells also causes numerous effects, including short-term replication, induction of IgE synthesis, and long-term proliferation when cultures are further supplemented with CD32 transfected L cells.

B7 (CD80) and B70 (CD86) are the second "group" of molecules which strongly mediate B and T cell interaction. These molecules, on B cells, interact with their ligands CD28 and CTLA-4 on T cells. These interactions are major co-stimulatory signals for activation of both B and T cells.

During the last 15 years, it has become apparent that these two pairs of surface molecules play fundamental functions in T cell and B cell activation

Numerous in vitro and in vivo experiments have demonstrated that these two pairs of molecules represent important targets for immunosuppression. See, e.g., Banchereau, et al. (1994) Ann. Rev. Immunol. 12:881-922; van Kooten, et al. (1996) Adv. Immunol. 61:1-77; Linsley and Ledbetter (1993) Ann. Rev. Immunol. 11:191-212).

Immunosuppression represents a very important immunological intervention to prevent and cure autoimmune diseases and graft rejection during transplantation. Immunosuppression can be achieved by a) anti-proliferative drugs (cyclophosphamide, 15-deoxyspergualin, et al.); b) glucocorticosteroids; c) inhibitors of intracellular signaling processes (e.g., cyclosporine); d) immunosuppressive cytokines (e.g., TGF-β, IL-10); e) specific tolerance induction by antigens; and f) inhibition of cell surface molecules involved in T and B lymphocyte activation, such as the CD40/CD40 ligand and B7/B70 with CD28/CTLA-4.

In 1995, another molecule called RP105 was cloned from mouse splenic cells. See Miyake, et al (1995) "RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family" J. Immunol. 154:3333-3340. Monoclonal antibody against RP105 induces strong proliferation of mouse B cells and protects mouse B cells from irradiation-induced apoptosis in a similar fashion to anti-CD40 antibody or CD40-ligand. See Miyake, et al. (1994) "Murine B cell proliferation and protection from apoptosis with an antibody against a 105 kDa molecule: Unresponsiveness of X-linked immunodeficient B cells" J. Exp. Med. 180:1217-1224.

The RP105 and its putative ligand may be a third pair of molecules that play key roles in the activation of T cells and B cells. However, until now, the human sequence has not been available, and its structure and activities undetermined. The present invention provides this and many other useful advances.

SUMMARY OF THE INVENTION

The present invention provides a composition selected from the group consisting of: an isolated or recombinant nucleic acid encoding human BAS-1 protein or fragment thereof; a substantially pure BAS-1 protein or peptide thereof; a fusion protein comprising BAS-1 protein sequence; and an antibody raised to a recombinant or purified primate BAS-1 protein.

In embodiments of the isolated or recombinant nucleic acid, the nucleic acid may comprise a sequence defined in SEQ ID NO: 1.

In protein embodiments, the protein may be a substantially pure BAS-1 protein or peptide thereof; be selected from the group consisting of: a protein or peptide from a primate, including a human; a protein or peptide comprising at least one polypeptide segment defined in SEQ ID NO: 2; a protein or peptide which exhibits a post-translational modification pattern distinct from a natural BAS-1 protein; and a protein lacking the intracellular domain of BAS-1; and may be a composition comprising the protein and a pharmaceutically acceptable carrier.

Antibody embodiments include those where the BAS-1 protein is a primate protein, including one from human; the antibody is raised against a peptide sequence defined in SEQ ID NO: 2; the antibody exhibits a Kd of at least about 10 μM; the antibody is a monoclonal antibody; or the antibody is labeled. Further embodiments include where the antibody induces strong proliferation of B cells; and/or protects B cells from irradiation- or steroid hormone-induced apoptosis.

The present invention also embraces kits, e.g., which comprise, e.g., a nucleic acid encoding a BAS-1 protein or peptide; a substantially pure BAS-1 protein or fragment; or an antibody or receptor which specifically binds a BAS-1 protein. A kit which includes the nucleic acid may comprise a coding sequence defined in SEQ ID NO: 1. For a kit which includes a protein or fragment, often

the polypeptide is selected from the group consisting of: a protein or peptide from a primate, including a human; a protein or peptide comprising at least one polypeptide segment defined in SEQ ID NO: 2; and a protein or peptide which exhibits a post-translational modification pattern distinct from a natural BAS-1 protein.

Another kit may include an antibody or receptor, wherein: the BAS-1 protein is a primate protein, including one from a human; the antibody is raised against a peptide sequence defined in SEQ ID NO: 2; the antibody exhibits a Kd of at least about 10 μM; the antibody is a monoclonal antibody; or the antibody is labeled.

The invention also provides a method of screening a sample for a binding partner for BAS-1 comprising the steps of producing a purified or recombinant BAS-1 protein, and screening in the sample for a specific binding of the binding partner to the BAS-1 protein. In certain embodiments, the sample comprises proteins derived from a T cell, a dendritic cell, including a follicular dendritic cell, or a stromal cell, including a fibroblast cell, an endothelial cell, and an epithelial cell. In other embodiments, the binding partner is an antibody, and the sample is a hybridoma supernatant.

Other aspects of the present invention include a method of modulating physiology or development of a cell comprising contacting the cell with an agonist or antagonist of a BAS-1 protein. Typically, the physiology is selected from: immunosuppression; activation of cytotoxic killing; modulation of cytokine production; or growth of a lymphoma. The antagonist often will be an antibody against a primate BAS-1 protein. And the method may include such in combination with a mediator of a signal through the antigen receptor, the CD40:CD40 ligand pathway, or the CD28/CTLA-4:B7/B70 pathway, e.g., anti-CD3, anti-CD40, anti-CD40 ligand, anti-CD28, anti-CTLA-4, anti-B7, anti-B70, or soluble portions of the appropriate surface signaling molecule.

DETAILED DESCRIPTION OF THE INVENTION

I. General

The present invention provides the amino acid sequence and DNA sequence of a human protein which exhibits properties of activation antigens. This protein is designated B cell Activation and Survival antigen-1 (BAS-1). The primate sequence described herein was obtained after a mouse gene was initially described. See Miyake, et al. (1995) J. Immunol. 154:3333-3340. Similar sequences for proteins in other primate species should also be available. The descriptions below are directed, for exemplary purposes, to the human BAS-1 natural allele described, but are likewise applicable to allelic and/or polymorphic variants, e.g., from other individuals, as well as splicing variants, e.g., natural forms.

These genes will allow isolation of other primate genes encoding proteins related to this, further extending the family beyond the specific embodiment described. The procedure is broadly set forth below.

Human B cell Activation and Survival Ag-1 (BAS-1), so named because of its biological activity. Monoclonal antibody against a mouse homolog, RP105, induces strong proliferation of mouse B cells and protects mouse B cells from irradiation-induced apoptosis in a similar fashion to anti-CD40 antibody or CD40-ligand.

The human BAS-1 cDNA was isolated using nucleic acid sequences based upon sequences from the mouse RP105. Analysis of the corresponding encoded protein indicates that the human BAS-1 is a member of the family of proteins which contain leucine-rich motifs. Other members of the family include the CD14-LPS receptor, FSH/LH/TSH receptors, and neurotropin receptors. These receptors appear to be involved in signal transduction.

Human BAS-1 cDNA was deduced using sequences from various regions of mouse RP105. A homology comparison between the mouse and human BAS-1 open reading frames exhibit approximately 67% DNA sequence identity and

approximately 73% amino acid sequence identity with the mouse RP105.

The human BAS-1 may also have functional roles outside the immune system, e.g., in developmental regulation in other cell types. See, e.g., Gilbert (1991) Developmental Biology (3d ed.), Sinauer Associates, Sunderland, MA; Browder, et al. (1991) Developmental Biology (3d ed.), Saunders, Philadelphia, PA.; Russo, et al. (1992) Development: The Molecular Genetic Approach, Springer-Verlag, New York, N.Y.; and Wilkins (1993) Genetic Analysis of Animal Development (2d ed.) Wiley-Liss, New York, N.Y. Identification of the ligand for human BAS-1 may help to address some of the questions raised by these observations.

II. Purified human BAS-1

SEQ ID NO: 1 discloses the nucleotide sequence of the cDNA and the corresponding amino acid sequence. The amino acid sequence is also set forth in SEQ ID NO: 2. The signal sequence appears to run from met (1) to val (20); an amino flanking region runs from cys (28) to leu (58), which contains two potential N-linked glycosylation sites on asparagine (residues 34, 53); the tandem repeats of leucine-rich motif run from thr (55) to tyr (592), which contain 9 potential sites for N-linked glycosylation on asparagine (residues 70, 78, 201, 234, 244, 394, 402, 451, and 573); the carboxy flanking region runs from leu (574) to cys (625); the transmembrane region runs from ala (629) to val (650); the intracellular region runs from lys (651) to phe (661), which contains two potential phosphorylation sites on tyr (652 and 658).

The following table shows the alignment of the leucine-rich repeats of the human BAS-1 protein shown in SEQ ID NO: 2.

Table 1

		C I I
consensus:		LXXLXLXXNXLXXLXXXXXXXLXX
1:	55-78	TEFLEFSFNFLPTIHNRTFSRLMN

2:	79-102	LTFLDLTRCQINWIPEDTFQSHHQ
3:	103-126	LSTLVLTGNPLIFMAETSLNGPKS
4:	127-150	LKHLFLIQTGISNLEFIPVHNLEN
5:	151-174	LESLYLGSNHISSIKFPKDFPARN
6:	175-198	LKVLDFQNNAIHYISREDMRSLEQ
7:	199-223	AINLSLNFNGNN-VKGIELGAFDSTV
8:	224-246	FQSLNFGGTPNL-SVIFNGLQNST
9:	247-275	TQSLWLGTFEDIDDEDISSAMLKGLCEMS
10:	276-299	VESLNLQEHRFSDISSTTFQCFTQ
11:	300-322	LQELDLTATHLKGLPSGMKG-LNL
12:	323-346	LKKLVLSVNHFDQLCQISAANFPS
13:	347-371	LTHLYIRGN-VKKLHLGVGC-LEKLGN
14:	372-397	LQTLDLSHNDIEASDCCSLQ-LKNLSH
15:	398-421	LQTLNLSHNEPLGLQSQAFKECPQ
16:	422-446	LELLDLAFTRLHINAPQSPFQNLHF
17:	447-470	LQVLNLTYCFLDTSNQHLLAGLPV
18:	471-497	LRHLNLKGNHFQDGTITKTNLLQTVGS
19:	498-521	LEVLILSSCGLLSIDQQAFHSLGK
20:	522-544	MSHVDLSHNSLTCDSIDSLSHLK
21:	545-568	GIYLNLAANSINIISPRLLPILSQ
22:	569-592	QSTINLSHNPLDCTCSNIHF-LTWY

As used herein, the term "human BAS-1" shall refer, when used in a protein context, to a protein having the amino acid sequence shown in SEQ ID NO: 2. The present invention also encompasses proteins comprising a substantial fragment thereof, e.g., mutants and polymorphic variants, along with a human derived polypeptide which exhibits the same biological function or interacts with human BAS-1 specific binding components. These binding components typically bind to a human BAS-1 with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Homologous proteins are found in species other than humans, e.g., primates.

The term "polypeptide" as used herein includes a fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least about 18 amino acids, more typically at least about 20 amino acids, usually at least about 22 amino acids, more usually at least about 24 amino acids, preferably at least about 26 amino acids,

more preferably at least about 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 33, 37, 41, 45, 49, 53, 57, etc.

The term "ligand" or "binding composition" refers to molecules that bind with specificity to BAS-1, e.g., in an antibody-antigen type fashion. Other interactions include, e.g., ligand-receptor type, or with compounds which associate with BAS-1, e.g., in a protein-protein interaction, either covalent or non-covalent. The molecule may be a polymer, or chemical reagent. No implication as to whether BAS-1 is either the ligand or the receptor of a ligand-receptor interaction is represented, other than the interaction exhibit specific affinity. A functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed.), Pergamon Press.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The electrolytes will usually approximate in situ physiological conditions, but may be modified to higher

or lower ionic strength where advantageous. The actual ions may be modified to conform to standard buffers used in physiological or analytical contexts.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions. In particular, it is believed that the natural protein is often linked to lipid via a PI linkage.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-([3-cholamidopropyl]dimethylammonio)-1-propane sulfonate), or in a low enough detergent concentration to not disrupt the tertiary structure of the protein.

Solubility is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.), W.H. Freeman; and Cantor and Schimmel (1980) Biophysical Chemistry, parts 1-3, W.H. Freeman & Co., San Francisco. As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. A soluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about

10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of the human BAS-1. It will embrace, e.g., 1-fold, 2-fold, and 3-fold conservative substitutions. Preferably the substitutions will be away from the conserved cysteines, and often will be in the regions away from the helical structural domains. Such variants may be useful to produce specific antibodies, and often will share many or all biological properties.

Amino acid sequence identity is determined by optimizing residue matches. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Similar amino acid sequences are intended to include natural allelic variations in each respective protein sequence. Typical homologous proteins or peptides will have from 85-100% identity (if gaps can be introduced), to 90-100% identity (if conservative substitutions are included) with the amino acid sequence of the human BAS-1 Identity measures will be at least about 85%, generally at least about 87%, often at least about 89%, typically at least about 91%, usually at least about 93%, more usually at least about 95%, preferably at least about 97%, and more preferably at least about 98%, and in particularly preferred embodiments, at least about 99% or more. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View,

CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

The isolated human BAS-1 DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications will result in novel DNA sequences which encode useful antigens, their derivatives, or proteins having similar or antagonist activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant BAS-1 derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant BAS-1" encompasses a polypeptide otherwise sharing important features of the human BAS-1 as set forth above, but having an amino acid sequence which differs from that of BAS-1 as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant BAS-1" is defined as having homology with an antigen defined in SEQ ID NO: 2, and as sharing various biological activities with those antigens. Similar concepts apply to different BAS-1 proteins, particularly those found in various other primates. As stated before, it is emphasized that descriptions are generally meant to encompass additional BAS-1 proteins, not limited solely to the human embodiment specifically discussed.

Although site specific mutation sites are predetermined, mutants need not be site specific. Human BAS-1 mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in

DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook, et al. (1989) and Ausubel, et al. (1987 and Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a BAS-1 polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of ligand-binding specificities and other functional domains.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

IV. Functional Variants

The blocking of physiological response to BAS-1 antigens may result from the inhibition of binding of the ligand to the BAS-1, likely through competitive inhibition. Thus, in vitro assays of the present invention will often use isolated protein, membranes from cells expressing a recombinant BAS-1, soluble fragments comprising ligand binding segments of these antigens, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or ligand mutations and modifications, e.g., ligand analogs.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the antigen or antigen fragments compete with a test compound for binding to the protein. In this manner, the antibodies can be used to detect the presence of any polypeptide which shares one or more binding sites of the antigen and can also be used to occupy binding sites on the protein that might otherwise be occupied by a ligand.

Additionally, neutralizing antibodies against the BAS-1 and soluble fragments of the BAS-1 which contain a high affinity ligand binding site, can be used to inhibit ligand function in tissues, e.g., tissues experiencing abnormal physiology.

"Derivatives" of the BAS-1 antigens include amino acid sequence mutants, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in the BAS-1 antigen amino acid side chains or at the N- or C- termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or

arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., human glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the BAS-1 antigens or fragments thereof with other proteins of polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred ligand derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between the BAS-1 antigens and other homologous or heterologous proteins are also provided. Homologous polypeptides may be fusions between different surface markers, resulting in, for instance, a hybrid protein exhibiting ligand specificity of one or more marker proteins. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of an antigen, e.g., a ligand-binding segment, so that the presence or location of a desired ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609, which is hereby incorporated herein by reference. Other gene

fusion partners include bacterial ß-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; and Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford.

This invention also contemplates the use of derivatives of the BAS-1 antigens other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are

useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of ligands or other binding ligands. For example, a BAS-1 antigen can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-BAS-1 antibodies or its ligand. The BAS-1 antigens can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

A solubilized BAS-1 antigen of this invention can be used as an immunogen for the production of antisera or antibodies specific for the antigen or any fragments thereof. The purified antigens can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the protein. In particular, the term "antibodies" also encompasses antigen binding fragments of natural antibodies. The purified BAS-1 can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of BAS-1 or cell fragments containing the antigen, both of which may be diagnostic of an abnormal or specific physiological or disease condition. Additionally, BAS-1 fragments may also serve as immunogens to produce the antibodies of the present invention, as described immediately below. For example, this invention contemplates antibodies raised against amino acid sequences of, or encoded by nucleotide sequences shown in SEQ ID NO: 1 or fragments thereof. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific fragments which are predicted to lie outside of the lipid bilayer. Additionally, various constructs may be produced from fusion of a membrane associating segment to the otherwise extracellular exposed portion of the molecule.

The present invention contemplates the isolation of additional closely related variants. It is highly likely that allelic variations exist in different individuals exhibiting, e.g., better than 90-97% identity to the embodiment described herein.

The invention also provides means to isolate a group of related antigens displaying both distinctness and similarities in structure, expression, and function. Elucidation of many of the physiological effects of the antigens will be greatly accelerated by the isolation and characterization of distinct species counterparts of the antigens. In particular, the present invention provides useful probes for identifying additional homologous genetic entities in different species.

The isolated genes will allow transformation of cells lacking expression of BAS-1, e.g., either species types or cells which lack corresponding antigens and exhibit negative background activity. Expression of transformed genes will allow isolation of antigenically pure cell lines, with defined or single specie variants. This approach will allow for more sensitive detection and discrimination of the physiological effects of any ligands. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

Dissection of the critical structural elements which effect the various differentiation functions provided by ligands is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et al. (1990) EMBO J. 9:4381-4390.

In particular, ligand binding segments can be substituted between species variants to determine what structural features are important in both ligand binding affinity and specificity. An array of different BAS-1 variants will be used to screen for ligands exhibiting

combined properties of interaction with different species variants.

Intracellular functions would probably involve segments of the antigen which are normally accessible to the cytosol. However, antigen internalization may occur under certain circumstances, and interaction between intracellular components and the designated "extracellular" segments may occur. The specific segments of interaction of BAS-1 with other intracellular components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components which may be isolatable by affinity methods.

Further study of the expression and control of BAS-1 antigens will be pursued. The controlling elements associated with the antigens exhibit differential developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest.

Structural studies of the BAS-1 antigens will lead to design of new ligands, particularly analogs exhibiting agonist or antagonist properties. This can be combined with previously described screening methods to isolate ligands exhibiting desired spectra of activities.

Expression in other cell types will often result in glycosylation differences in a particular antigen. Various species variants may exhibit distinct functions based upon structural differences other than amino acid sequence. Differential modifications may be responsible for differential function, and elucidation of the effects are now made possible.

Thus, the present invention provides important developmental antigens and reagents developed from them. Although the foregoing description has focused primarily upon the human BAS-1, those of skill in the art will immediately recognize that the invention encompasses

other BAS-1 antigens, e.g., primate and other mammalian species variants.

V. Antibodies

Antibodies can be raised to the various allelic variants of BAS-1 antigens and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to BAS-1 in either their active forms or in their inactive forms. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of BAS-1 can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective BAS-1, or screened for agonistic or antagonistic ligand activity. These monoclonal antibodies will usually bind with at least a K_D of better than about 1 mM, more usually better than about 300 µM, typically better than about 10 µM, more typically better than about 30 µM, preferably better than about 10 µM, and more preferably better than about 3 µM, e.g., 1 µM, 300 nM, 100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, etc.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to BAS-1 and inhibit ligand binding or inhibit the ability of a ligand to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to the antigen, the cell itself is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can bind to the BAS-1

without inhibiting ligand binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying BAS-1 or its ligands.

BAS-1 fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. A BAS-1 and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York, and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York, for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated. Alternatively, cells may be collected for producing hybridomas.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of

hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, SEPHADEX, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby the purified BAS-1 protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against a BAS-1 antigen will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

VI. Nucleic Acids

The human BAS-1 probe, or fragments thereof, will be used to identify or isolate nucleic acids encoding homologous proteins from other species, or other related proteins in the same or another species.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding BAS-1 polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide which is capable of hybridizing under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact BAS-1, or fragment, and have an amino acid sequence encoded by a nucleic acid shown in SEQ ID NO: 1. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encodes a protein which is homologous to a BAS-1 or which was isolated using cDNA encoding human BAS-1 as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and flanking genomic sequences from the originating species. The invention embraces a nucleic acid sequence which has

been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing , e.g., a restriction or sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic

acids which, by genetic code redundancy, encode similar polypeptides to fragments of these antigens, and fusions of sequences from various different species variants.

A "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 20 nucleotides, more generally at least about 23 nucleotides, ordinarily at least about 26 nucleotides, more ordinarily at least about 29 nucleotides, often at least about 32 nucleotides, more often at least about 35 nucleotides, typically at least about 38 nucleotides, more typically at least about 41 nucleotides, usually at least about 44 nucleotides, more usually at least about 47 nucleotides, preferably at least about 50 nucleotides, more preferably at least about 53 nucleotides, and in particularly preferred embodiments will be at least about 56 or more nucleotides, e.g., 60, 75, 100, 150, 200, 250, 300, etc.

A DNA which codes for a BAS-1 protein will be particularly useful to identify genes, mRNA, and cDNA species which code for related or homologous antigens, as well as DNAs which code for homologous proteins from different species. Various BAS-1 proteins should be similar in sequence and are encompassed herein. However, even proteins that have a more distant evolutionary relationship to the BAS-1 can readily be isolated using these sequences if they exhibit sufficient similarity. Primate BAS-1 proteins are of particular interest.

This invention further encompasses recombinant DNA molecules and fragments having a DNA sequence identical to or highly homologous to the isolated DNAs set forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication. Alternatively, recombinant clones derived from the genomic sequences, e.g., containing introns, will be useful for transgenic studies, including, e.g., transgenic cells and organisms, and for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology Academic Press, San

Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (1987)(ed.) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Homologous nucleic acid sequences, when compared, exhibit significant sequence similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. The hybridization conditions are described in greater detail below.

Substantial identity in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least about 56%, more generally at least about 59%, ordinarily at least about 62%, more ordinarily at least about 65%, often at least about 68%, more often at least about 71%, typically at least about 74%, more typically at least about 77%, usually at least about 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides. Alternatively, substantial identity exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from SEQ ID NO: 1. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at

least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides, e.g., 125, 150, 200, 250, 300, etc.

Stringent conditions, in referring to identity in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 500 mM, usually less than about 350 mM, more usually less than about 200 mM, typically less than about 150 mM, preferably less than about 100 mM, and more preferably less than about 50 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370.

BAS-1 from other human subjects can be cloned and isolated by hybridization or PCR. Alternatively, preparation of an antibody preparation which exhibits less allelic specificity may be useful in expression cloning approaches. Allelic variants may be characterized using, e.g., a combination of redundant PCR and sequence analysis, e.g., using defined primers, thereby providing information on allelic variation in a human population.

VII. Making BAS-1; Mimetics

DNA which encodes the BAS-1 antigen or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries

prepared from a wide variety of cell lines or tissue samples.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length antigen or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies. Each antigen or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially purified to be free of protein or cellular contaminants, e.g., those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The antigen, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired antigen gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes a human BAS-1 antigen, or a fragment thereof encoding a biologically active polypeptide. The DNA can

be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a human BAS-1 antigen in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the antigen is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the antigen or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of the human BAS-1 gene or its fragments into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriquez, et al. (1988)(eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, MA.

Transformed cells are cells, preferably mammalian, that have been transformed or transfected with human BAS-1 vectors constructed using recombinant DNA techniques. Transformed host cells usually express the antigen or its fragments, but for purposes of cloning, amplifying, and

manipulating its DNA, do not need to express the protein. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the protein to accumulate in the culture. The protein can be recovered, either from the culture or from the culture medium.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the human BAS-1 antigens or its fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter

(pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, Chapter 10, pp. 205-236.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with human BAS-1 antigen sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the desired protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are the preferred host cells for expression of the functionally active human BAS-1 antigen protein. In principle, many higher eukaryotic tissue culture cell lines are workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred, in that the processing, both cotranslationally and posttranslationally. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful

cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

It will often be desired to express a human BAS-1 antigen polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the BAS-1 antigen gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable or approximated in prokaryote or other cells.

The BAS-1 antigen might also be produced in a form which is phosphatidyl inositol (PI) linked, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991)

J. Cell Biol. 114:1275-1283. Alternatively, purification segments may be engineered into the sequence, e.g., at the N-terminus or C-terminus, to assist in the purification or detection of the protein product. Means to remove such segments may also be engineered, e.g., protease cleavage sites.

Now that the entire sequence is known, the human BAS-1 antigens, fragments, or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes.

The human BAS-1 antigens, fragments, or derivatives are suitably prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that are not being used in the coupling reaction must be protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier is not particularly limited as long as it has a binding capability to a reactive carboxyl group. Examples of such insoluble carriers include halomethyl

resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl resins, phenol resins, tert-alkyloxycarbonyl-hydrazidated resins, and the like.

An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield, et al. (1963) in J. Am. Chem. Soc. 85:2149-2156.

The prepared antigen and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, for example, by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The human BAS-1 antigens of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of the protein purification techniques disclosed herein or by the use of the antibodies herein described in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of small cell lung cancer cells, lysates of other cells expressing the BAS-1 antigens, or lysates or supernatants of cells producing the BAS-1 antigens as a result of DNA techniques, see below.

VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in the general description for developmental or physiological abnormalities, or below in the description of kits for diagnosis.

This invention also provides reagents with significant therapeutic value. The human BAS-1

(naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to human BAS-1, should be useful in the treatment of conditions associated with abnormal B cell response, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression or abnormal triggering of BAS-1 should be a likely target for an agonist or antagonist of the antigen. BAS-1 likely plays a role in activation or regulation of B cells, which affect immunological responses, e.g., autoimmune disorders or allergic responses.

In addition, the BAS-1:BAS-1 binding partner interaction may be involved in T:B cell interactions that permit the activtion, proliferation, and/or differentiation of naive B cells as observed in the hyper-IgM syndrome. If so, treatment may result form interference with the BAS-1:BAS-1 binding partner signal transduction. Blocking of the signal may be effected, e.g., by soluble BAS-1 or antibodies to BAS-1.

The BAS-1:BAS-1 binding partner interaction may also be involved in initiating and/or regulation of the massive proliferation of B cell blasts and centroblasts in the dark zone of the germinal centers. See, e.g., Banchereau and Rosset (1992) Adv. Immunol. 125:868-877. The cell surface interactions involved in the signal to initiate and/or regulate the Ig somatic mutation process may also involve BAS-1, and may be regulated by agonistic or antagonistic intervention.

Other abnormal developmental conditions are known in each of the cell types shown to possess BAS-1 mRNA by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y. For example, therapeutic

immunosuppression may be achieved by blocking T lymphocyte and B lymphocyte interaction through this molecule. It will represent an important therapy for controlling autoimmune diseases and graft rejection during transplantation. Alternatively, anti-B cell tumor therapy may be achieved by blocking the growth and survival effects of BAS-1 on B cells. The blockage may be effected by blocking binding compositions, e.g., neutralizing antibodies, or by molecules which interfere with the interaction of BAS-1 with its counterreceptor. Soluble BAS-1 may block the counterreceptor interaction.

Recombinant BAS-1 or BAS-1 antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof which are not complement binding.

Drug screening using BAS-1 or fragments thereof can be performed to identify compounds having binding affinity to BAS-1, including isolation of associated components. Subsequent biological assays can then be utilized to determine whether the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of a ligand. Likewise, a compound having intrinsic stimulating activity can activate the antigen and is thus an agonist in that it simulates the activity of BAS-1. This invention further contemplates the therapeutic use of antibodies to BAS-1 as antagonists. This approach should be particularly useful with other BAS-1 species variants.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site,

physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

Human BAS-1, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both

pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for topical, oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Both the naturally occurring and the recombinant form of the BAS-1 antigens of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble BAS-1 as provided by this invention.

For example, antagonists can normally be found once the BAS-1 has been structurally defined. Testing of potential ligand antagonists is now possible upon the development of highly automated assay methods using a purified BAS-1. In particular, new agonists and antagonists will be discovered by using screening techniques made available herein. Of particular importance are compounds found to have a combined binding affinity for multiple BAS-1 proteins, e.g., compounds which can serve as antagonists for allelic variants of BAS-1.

Moreover, since the signaling through the BAS-1:BAS-1 binding partner may function in combination with other signals, e.g., the CD28/CTLA-4:B7/B70 and/or the CD40:CD40 ligand pathways, combination compositions or combination therapy with such pathways will also be considered. Thus, antagonism of multiple signal pathways, or stimulation with multiple pathways may be useful.

This invention is particularly useful for screening compounds by using the recombinant antigens in any of a variety of drug screening techniques. The advantages of using a recombinant protein in screening for specific ligands include: (a) improved renewable source of the BAS-1 from a specific source; (b) potentially greater number of antigen molecules per cell giving better signal to noise ratio in assays; and (c) species variant specificity (theoretically giving greater biological and disease specificity).

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the BAS-1. Cells may be isolated which express a BAS-1 in isolation from others. Such cells, either in viable or fixed form, can be used for standard antigen/ligand binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses. Competitive assays are particularly useful, where the cells (source of BAS-1) are contacted and incubated with a labeled ligand having known binding affinity to the antigen, such as ^{125}I-ligand, and a test compound whose binding affinity to the BAS-1 is being measured. The bound ligand and free ligand are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled ligand binding measured. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure

such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on BAS-1 mediated functions, e.g., second messenger levels, i.e., Ca^{++}; cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

Another method utilizes membranes from transformed eukaryotic or prokaryotic host cells as the source of the human BAS-1. These cells are stably transformed with DNA vectors directing the expression of human BAS-1 antigen. Essentially, the membranes would be prepared from the cells and used in any receptor/ligand binding assay such as the competitive assay set forth above.

Still another approach is to use solubilized, unpurified or solubilized, purified BAS-1 from transformed eukaryotic or prokaryotic host cells. This allows for a "molecular" binding assay with the advantages of increased specificity, the ability to automate, and high drug test throughput.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to human BAS-1 and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al. (1991). Then all the pins are reacted with solubilized, unpurified or solubilized, purified BAS-1, and washed. The next step involves detecting bound BAS-1.

Rational drug design may also be based upon structural studies of the molecular shapes of the BAS-1 and other effectors or ligands. Effectors may be other

proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the antigen. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

Purified BAS-1 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to these antigens can be used as capture antibodies to immobilize the respective BAS-1 on the solid phase.

IX. Kits

This invention also contemplates use of BAS-1 proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of BAS-1 or a ligand. Typically the kit will have a compartment containing either a defined BAS-1 peptide or gene segment or a reagent which recognizes one or the other.

A kit for determining the binding affinity of a test compound to a BAS-1 would typically comprise a test compound; a labeled compound, for example a ligand or antibody having known binding affinity for the BAS-1; a source of BAS-1 (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the BAS-1. Once compounds are screened, those having suitable binding affinity to the BAS-1 can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists. The availability of recombinant BAS-1 polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, a BAS-1 in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for the BAS-1, a source of BAS-1 (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, for example, a solid phase for immobilizing the BAS-1. Compartments containing reagents, and instructions, will normally be provided.

One method for determining the concentration of BAS-1 in a sample would typically comprise the steps of: (1) preparing membranes from a sample comprised of a BAS-1 source; (2) washing the membranes and suspending them in a buffer; (3) solubilizing the BAS-1 by incubating the membranes in a culture medium to which a suitable detergent has been added; (4) adjusting the detergent concentration of the solubilized BAS-1; (5) contacting and incubating said dilution with radiolabeled ligand to form complexes; (6) recovering the complexes such as by filtration through polyethyleneimine treated filters; and (7) measuring the radioactivity of the recovered complexes.

Antibodies, including antigen binding fragments, specific for human BAS-1 or BAS-1 fragments are useful in diagnostic applications, e.g., to detect the presence of elevated levels of BAS-1 and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the BAS-1 in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-ligand complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and

which recognizes the antibody to a BAS-1 or to a particular fragment thereof. These assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a human BAS-1, as such may be diagnostic of various abnormal states. For example, overproduction of BAS-1 may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in proliferative cell conditions such as cancer or abnormal differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody, or labeled BAS-1 is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Any of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the ligand, test compound, BAS-1, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No.

3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Both of the patents are incorporated herein by reference. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The BAS-1 can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the BAS-1 to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of antigen/ligand complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

The methods for linking proteins or their fragments to the various labels have been extensively reported in the literature. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of polynucleotide or oligonucleotide sequences taken from the sequence of a BAS-1. These sequences can be

used as probes for detecting levels of the antigen in samples from patients suspected of having an abnormal condition, e.g., cancer or developmental problem. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P. However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in any conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

X. Ligand or Counterreceptor

The description of the BAS-1 protein herein provides means to identify a counterreceptor or ligand. Such ligand or counterreceptor should bind specifically to the BAS-1 with reasonably high affinity. Various constructs are made available which allow either labeling of the BAS-1 to detect its partner. For example, directly labeling BAS-1, fusing onto it markers for secondary labeling, e.g., FLAG or other epitope tags, Ig domain fusions, etc., will allow detection of binding partners. This can be histological, as an affinity method for biochemical purification, or labeling or selection in an expression cloning approach. A two-hybrid selection system may also be applied making appropriate constructs with the available BAS-1 sequences. See, e.g., Fields and Song (1989) Nature 340:245-246.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

I. General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of

protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Computer sequence analysis is performed, e.g., using available software programs, including those from the GCG (U. Wisconsin) and GenBank sources. Public sequence databases were also used, e.g., from GenBank and others.

II. Amplification of human BAS-1 fragment by PCR

Two primers were designed according to the mouse RP105 sequences. See Miyake, et al (1995) J. Immunol. 154:3333-3340. Nucleotide designations are counted from a CTT sequence therein. 5' primer A (TTG...) corresponds to 24 nucleotides from position 162 to 185 and 3' primer F (...TTC) corresponds to 24 nucleotides from position 1462 to 1485. No PCR products were obtained from human tonsillar B cells.

Three 5' primers were made: primer B (ACA...) covering nucleotide positions 321-344, primer C (AAG...) covering nucleotide positions 568-591, and primer D (TCT...) covering nucleotide positions 859-882. Three other 3' primers were also made: primer G (... TTA) covering nucleotide position 1765-1788, primer H (...GAA) covering nucleotide position 2024-2047, and primer E (...TGG) covering nucleotide position 1164-1187.

To increase the chances of obtaining PCR products from human tonsillar B cells, the PCR reactions were performed by using one 5' primer with the combination of the four 3' primers. Out of these four groups of PCR reactions, only one clear product of 300 bp was obtained,

48

which spanned from 5' primer D to 3' primer E. This product was purified, subcloned into pCR™ vector (Invitrogen, San Diego CA), and then sequenced. See SEQ ID NO: 1.

According to the 300 bp human sequence, 3 primers were designed: 5' primers K; 3' primers I and L. Using a similar PCR method, another 300 bp human fragment was obtained with the combination of primers C and I (see SEQ ID NO: 1).

III. Tissue distribution of human BAS-1

The PCR product amplified by the primer pair D-E (see SEQ ID NO: 1) was used as a probe (D-E probe) to study the tissue distribution of BAS-1 in human tissues. A 2.6 kb mRNA was detected in human spleen and at a lower level in human heart by Northern blot. This was not readily detected in human brain, thymus, lung, liver, skeletal muscle, kidney, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocyte. In addition, the 2.6 kb message was detected by Northern blot in a sIgD+ lymphoma cell line B104 and at a lower level in a Burkitt's lymphoma cell line BL2, but not in kidney epithelial carcinoma cell line CHA, lung epithelial carcinoma cell line MRC-5, EBV cell line JY, or monocyte cell line U937.

By PCR, human BAS-1 message RNA was found in $sIgD^{+}CD38^{-}$ naive B cells, $sIgD^{+}CD38^{+}$ germinal center B cells, $sIgD^{-}CD38^{+}$ germinal center B cells, $sIgD^{-}CD38^{-}$ memory B cells, $sCD38^{++}CD20^{low}$ plasma cells, dendritic cells generated from CD14+ monocytes cultured with GM-CSF plus IL-4, and dendritic cells generated from CD34+ cord blood cells cultured with GM-CSF plus TNF-α. This message was not detectable by PCR in the human T cell line MT9.

IV. Screening of a plasmid cDNA library derived from a human $sIgD^{+}$ lymphoma cell line B104

From the result of Northern blot analysis, BAS-1 expression was detected in 6 μg of poly-A mRNA of the B104 cell line. Accordingly, a B104 cDNA library was

constructed in pSPORT1 plasmid (Gibco Life Technologies, Cergy Pontoise, France). After screening of 150,000 bacteria colonies, no positive clone was obtained by using the D-E probe. This may reflect that the expression of BAS-1 in human cells may be very low.

V. Screening a λgt10 bacteriophage library of human spleen

In order to screen more clones, a λgt10 bacteriophage library of human spleen (Clontech) was used. After screening 2.4 x 10^6 clones, 8 positive clones were identified. These clones were isolated, purified, and subcloned into pME18S plasmid (DNAX, Palo Alto, CA). After sequencing, two clones S2 and L1 were found to represent full length human BAS-1 (see SEQ ID NO: 1). The full length human BAS-1 cDNA isolated contains about 2635 bp, and encodes a protein of about 661 aa. This protein product contains about 11 potential sites for glycosylation and about 22 leucine-rich repeat motifs. The coding region of this human BAS-1 cDNA shares about 72% homology with the coding region of the mouse RP105 sequence, and this complete BAS-1 cDNA shares about 67% homology with the complete mouse cDNA sequence. The amino acid homology between human BAS-1 and mouse RP105 is about 73.5%.

VI. Expression of human BAS-1 protein

All constructions were made by eliminating the 5' and 3' non-coding region and by adding the Marylin Kozak (Ref: J. Biol. Chem. 266: 19867, 1991) consensus sequence (ACCATGG; SEQ ID NO: 3) to enhance the translational level.

Soluble BAS-1-FLAG protein has been transiently expressed of in Cos 7 cells, as follows. A recombinant form of BAS-1 displaying the FLAG peptide at the carboxy terminus (Hoppe, et al. (1988) Biotechnology 6:1205-1210) was introduced into the expression vector pME18S and subsequently transfected into Cos-7 cells by electroporation. Electroporated cells were grown in DMEM

medium supplemented either with 1% Nutridoma HU (Boehringer Mannheim, Mannheim, Germany) or DMEM medium alone for 5 days.

VII. Purification of soluble BAS-1 Flag protein

Typically, 280 ml of supernatant containing soluble BAS-1 FLAG was passed on a 20 ml column of Cu^{++} ions attached to a Chelating Sepharose Fast Flow matrix (Pharmacia, Upsalla, Sweden). After washing with 16 volumes of binding buffer (His-Bind Buffer kit, Novagen, Madison, WI), the proteins retained by the metal ions were eluted with a gradient of 20-100 mM Imidazole. The content of human BAS-1 FLAG in the eluted fractions was determined by dot blot using the anti-FLAG monoclonal antibody M2 (Eastman Kodak, New Haven, CT) and by Coomassie blue and silver staining of reducing SDS-PAGE. The BAS-1 FLAG protein containing fractions were then pooled and dialyzed against PBS. By Western blot, the enriched human BAS-1 correspond to about 95 and 97 kd proteins, as predicted from the amino acid sequences.

VIII. Stable expression of membrane BAS-1 in NIH-3T3 cells

A native membrane form was subcloned into the expression vector pMAMneo (Clontech, Palo Alto, California), which contains the RSV-LTR enhancer linked to the dexamethasone-inducible MMTV-LTR promoter. This construct was then transfected into NIH-3T3 cells by electroporation. Transfected NIH-3T3 cells were seeded in selective 0.5 mg/ml Geneticin (G418) (Boehringer-Mannheim, Mannheim, Germany) DMEM supplemented with 10% Fetal Calf Serum.

Biochemical characterization of membrane BAS-1 protein in these stable transfected NIH-3T3 cells has been with metabolic labeling. Cells were then cultivated for 24 hours in DMEM medium supplemented with 10% Fetal Calf Serum and 1 µM final dexamethasone (Sigma, Saint Quentin Fallavier, France). Cells were then incubated with ^{35}S-Met and ^{35}S-Cys during the last 12 hours in

order to label cellular proteins. Analysis of the proteins under reducing conditions on SDS-PAGE showed the predicted 110 kDa protein in the lysate of the transfected NIH-3T3 cells, but not in the lysate of untransfected NIH-3T3 cells.

IX. Preparation of antibodies specific for BAS-1

Inbred Balb/c mice have been immunized intraperitoneally with recombinant forms of the human protein. The first received the purified soluble BAS-1 FLAG and the second received the stable transfected NIH-3T3 cells. Animals are boosted at appropriate time points with protein, with or without additional adjuvant, to further stimulate antibody production. Serum is collected, or hybridomas produced with harvested spleens.

Alternatively, Balb/c mice are immunized with cells transformed with the gene or fragments thereof, either endogenous or exogenous cells, or with isolated membranes enriched for expression of the antigen. Serum is collected at the appropriate time, typically after numerous further administrations. Various gene therapy techniques may be useful, e.g., in producing protein in situ, for generating an immune response.

Monoclonal antibodies may be made. For example, splenocytes are fused with an appropriate fusion partner and hybridomas are selected in growth medium by standard procedures. Hybridoma supernatants are screened for the presence of antibodies which bind to the human BAS-1, e.g., by ELISA or other assay. Antibodies which specifically recognize human BAS-1 but not species variants may also be selected or prepared.

In another method, synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a

substrate for panning methods. Nucleic acids may also be introduced into cells in an animal to produce the antigen, which serves to elicit an immune response. See, e.g., Wang, et al. (1993) Proc. Nat'l. Acad. Sci. 90:4156-4160; Barry, et al. (1994) BioTechniques 16:616-619; and Xiang, et al. (1995) Immunity 2: 129-135.

X. Large scale production of BAS-1 in NS0 cells

Large quantities of soluble BAS-1 and soluble BAS-1 FLAG can be produced from stable transformants of NS0 cells prepared, e.g., according to a methodology developed by Celltech (Slough, Berkshire, UK; International Patent Applications WO86/05807, WO87/04462, WO89/01036 and WO89/10404). Both BAS-1 and BAS-1-FLAG were subcloned into pEE12 and subsequently transfected into NS0 cells by electroporation. Transfected NS0 cells were seeded in selective glutamine-free DMEM supplemented with 10% Fetal Calf Serum as described in Celltech's protocol. Supernatants from the best producing lines are used in biological assays and purification of soluble BAS-1 and soluble BAS-1-FLAG.

Other expression systems can be used to produce large quantities of recombinant proteins.

XI. Production of fusion proteins with BAS-1

Various fusion constructs are made with the BAS-1 extracellular domain. This portion of the gene is fused to another protein, e.g., a FLAG epitope tag, an Ig domain, or to a two hybrid system construct. See, e.g., Fields and Song (1989) Nature 340:245-246.

The epitope tag may be used in an expression cloning procedure with detection with anti-FLAG antibodies to detect a binding partner, e.g., ligand for the BAS-1. Alternatively, the Ig domain may be used to purify using antigen-like affinity for ligand. The two hybrid system may also be used to isolate proteins which specifically bind to BAS-1.

XII. Mapping of human BAS-1 by in situ hybridization

Chromosome spreads were prepared. In situ hybridization was performed on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 5-bromodeoxyuridine was added for the final seven hours of culture (60 μg/ml of medium), to ensure a posthybridization chromosomal banding of good quality.

A 655 base pair PCR fragment, amplified with the help of primers, e.g., corresponding to nucleotides 395-411 and 1027-1050, on total B cells cDNA template, was cloned into an appropriate vector. The vector was labeled by nick-translation with ^{3}H. The radiolabeled probe was hybridized to metaphase spreads at final concentration of 200 ng/ml of hybridization solution as described in Mattei, et al. (1985) Hum. Genet. 69:327-331.

After coating with nuclear track emulsion (KODAK NTB_2), slides were exposed for 18 days at 4° C. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphase photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa (FPG) method and metaphases rephotographed before analysis.

In 100 metaphase cells examined after in situ hybridization, 20% of silver grains were located on chromosome 5. The distribution of grains was not random, 67% of them mapped to the q11.2-q13.1 region of chromosome 5 long arm. This maps the human BAS-1 to the 5q11.2-q13.1 region of the human genome.

XIII. Isolation of a Receptor for Human BAS-1

A human BAS-1 can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

The binding composition is used to screen an expression library made from a cell line which expresses a binding partner, i.e. receptor. Standard staining techniques are used to detect or sort intracellular or surface expressed receptor, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-2832.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at 2-3 x 10^5 cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

On day 1 for each sample, prepare 0.5 ml of a solution of 66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in serum free DME. For each set, a positive control is prepared, e.g., of human BAS-1-FLAG cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin (0.1%) with 32 µl/ml of 1 M NaN_3 for 20 min. Cells are then washed with HBSS/saponin 1X. Add human BAS-1 or BAS-1/antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. If appropriate, add first antibody for 30 min. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop

solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min, which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H_2O_2 per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air dry for a few minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90° C.

Evaluate positive staining of pools and progressively subclone to isolation of single genes responsible for the binding.

Alternatively, BAS-1 reagents are used to affinity purify or sort out cells expressing a receptor. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound receptor by panning. The receptor cDNA is constructed as described above. The ligand can be immobilized and used to immobilize expressing cells. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence of a BAS-1 fusion construct, or by use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

Phage expression libraries can be screened by human BAS-1. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate clones.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the

56

appended claims, along with the full scope of equivalents to which such claims are entitled.

57

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Schering Corporation

(ii) TITLE OF INVENTION: HUMAN B CELL ANTIGENS; RELATED REAGENTS

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Schering-Plough Corporation
(B) STREET: 2000 Galloping Hill Road
(C) CITY: Kenilworth
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07033

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: 7.5.3

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

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/649,553
(B) FILING DATE: 17-MAY-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Cynthia L. Foulke, Esq.
(B) REGISTRATION NUMBER: 32,364
(C) REFERENCE/DOCKET NUMBER: DX0580PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 908-298-2987
(B) TELEFAX: 908-298-5388

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2635 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 97..2082

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

ATTCCGGCGG CTGCCAAATT GCCAGGGCCT TCACAGTTTG ATTCCATTTC TCAGCTCCAA
60

58

```
GCATTAGGTA AACCCACCAA GCAATCCTAG CCTGTG ATG GCG TTT GAC GTC AGC
114
                                        Met Ala Phe Asp Val Ser
                                          1               5

TGC TTC TTT TGG GTG GTG CTG TTT TCT GCC GGC TGT AAA GTC ATC ACC
162
Cys Phe Phe Trp Val Val Leu Phe Ser Ala Gly Cys Lys Val Ile Thr
             10                  15                  20

TCC TGG GAT CAG ATG TGC ATT GAG AAA GAA GCC AAC AAA ACA TAT AAC
210
Ser Trp Asp Gln Met Cys Ile Glu Lys Glu Ala Asn Lys Thr Tyr Asn
         25                  30                  35

TGT GAA AAT TTA GGT CTC AGT GAA ATC CCT GAC ACT CTA CCA AAC ACA
258
Cys Glu Asn Leu Gly Leu Ser Glu Ile Pro Asp Thr Leu Pro Asn Thr
     40                  45                  50

ACA GAA TTT TTG GAA TTC AGC TTT AAT TTT TTG CCT ACA ATT CAC AAT
306
Thr Glu Phe Leu Glu Phe Ser Phe Asn Phe Leu Pro Thr Ile His Asn
 55                  60                  65                  70

AGA ACC TTC AGC AGA CTC ATG AAT CTT ACC TTT TTG GAT TTA ACT AGG
354
Arg Thr Phe Ser Arg Leu Met Asn Leu Thr Phe Leu Asp Leu Thr Arg
                 75                  80                  85

TGC CAG ATT AAC TGG ATA CAT GAA GAC ACT TTT CAA AGC CAT CAT CAA
402
Cys Gln Ile Asn Trp Ile His Glu Asp Thr Phe Gln Ser His His Gln
             90                  95                  100

TTA AGC ACA CTT GTG TTA ACT GGA AAT CCC CTG ATA TTC ATG GCA GAA
450
Leu Ser Thr Leu Val Leu Thr Gly Asn Pro Leu Ile Phe Met Ala Glu
         105                 110                 115

ACA TCG CTT AAT GGG CCC AAG TCA CTG AAG CAT CTT TTC TTA ATC CAA
498
Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys His Leu Phe Leu Ile Gln
     120                 125                 130

ACG GGA ATA TCC AAT CTC GAG TTT ATT CCA GTG CAC AAT CTG GAA AAC
546
Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro Val His Asn Leu Glu Asn
135                 140                 145                 150

TTG GAA AGC TTG TAT CTT GGA AGC AAC CAT ATT TCC TCC ATT AAG TTC
594
Leu Glu Ser Leu Tyr Leu Gly Ser Asn His Ile Ser Ser Ile Lys Phe
                 155                 160                 165

CCC AAA GAC TTC CCA GCA CGG AAT CTG AAA GTA CTG GAT TTT CAG AAT
642
Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys Val Leu Asp Phe Gln Asn
             170                 175                 180

AAT GCT ATA CAC TAC ATC TCT AGA GAA GAC ATG AGG TCT CTG GAG CAG
690
Asn Ala Ile His Tyr Ile Ser Arg Glu Asp Met Arg Ser Leu Glu Gln
         185                 190                 195
```

```
GCC ATC AAC CTA AGC CTG AAC TTC AAT GGC AAT AAT GTT AAA GGT ATT
738
Ala Ile Asn Leu Ser Leu Asn Phe Asn Gly Asn Asn Val Lys Gly Ile
    200                 205                 210

GAG CTT GGG GCT TTT GAT TCA ACG GTC TTC CAA AGT TTG AAC TTT GGA
786
Glu Leu Gly Ala Phe Asp Ser Thr Val Phe Gln Ser Leu Asn Phe Gly
215                 220                 225                 230

GGA ACT CCA AAT TTG TCT GTT ATA TTC AAT GGT CTG CAG AAC TCT ACT
834
Gly Thr Pro Asn Leu Ser Val Ile Phe Asn Gly Leu Gln Asn Ser Thr
                235                 240                 245

ACT CAG TCT CTC TGG CTG GGA ACA TTT GAG GAC ATT GAT GAC GAA GAT
882
Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu Asp Ile Asp Asp Glu Asp
            250                 255                 260

ATT AGT TCA GCC ATG CTC AAG GGA CTC TGT GAA ATG TCT GTT GAG AGC
930
Ile Ser Ser Ala Met Leu Lys Gly Leu Cys Glu Met Ser Val Glu Ser
        265                 270                 275

CTC AAC CTG CAG GAA CAC CGC TTC TCT GAC ATC TCA TCC ACC ACA TTT
978
Leu Asn Leu Gln Glu His Arg Phe Ser Asp Ile Ser Ser Thr Thr Phe
    280                 285                 290

CAG TGC TTC ACC CAA CTC CAA GAA TTG GAT CTG ACA GCA ACT CAC TTG
1026
Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp Leu Thr Ala Thr His Leu
295                 300                 305                 310

AAA GGG TTA CCC TCT GGG ATG AAG GGT CTG AAC TTG CTC AAG AAA TTA
1074
Lys Gly Leu Pro Ser Gly Met Lys Gly Leu Asn Leu Leu Lys Lys Leu
                315                 320                 325

GTT CTC AGT GTA AAT CAT TTC GAT CAA TTG TGT CAA ATC AGT GCT GCC
1122
Val Leu Ser Val Asn His Phe Asp Gln Leu Cys Gln Ile Ser Ala Ala
            330                 335                 340

AAT TTC CCC TCC CTT ACA CAC CTC TAC ATC AGA GGC AAC GTG AAG AAA
1170
Asn Phe Pro Ser Leu Thr His Leu Tyr Ile Arg Gly Asn Val Lys Lys
        345                 350                 355

CTT CAC CTT GGT GTT GGC TGC TTG GAG AAA CTA GGA AAC CTT CAG ACA
1218
Leu His Leu Gly Val Gly Cys Leu Glu Lys Leu Gly Asn Leu Gln Thr
    360                 365                 370

CTT GAT TTA AGC CAT AAT GAC ATA GAG GCT TCT GAC TGC TGC AGT CTG
1266
Leu Asp Leu Ser His Asn Asp Ile Glu Ala Ser Asp Cys Cys Ser Leu
375                 380                 385                 390

CAA CTC AAA AAC CTG TCC CAC TTG CAA ACC TTA AAC CTG AGC CAC AAT
1314
Gln Leu Lys Asn Leu Ser His Leu Gln Thr Leu Asn Leu Ser His Asn
```

60

395 400 405

GAG CCT CTT GGT CTC CAG AGT CAG GCA TTC AAA GAA TGT CCT CAG CTA
1362
Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe Lys Glu Cys Pro Gln Leu
410 415 420

GAA CTC CTC GAT TTG GCA TTT ACC CGC TTA CAC ATT AAT GCT CCA CAA
1410
Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu His Ile Asn Ala Pro Gln
425 430 435

AGT CCC TTC CAA AAC CTC CAT TTC CTT CAG GTT CTG AAT CTC ACT TAC
1458
Ser Pro Phe Gln Asn Leu His Phe Leu Gln Val Leu Asn Leu Thr Tyr
440 445 450

TGC TTC CTT GAT ACC AGC AAT CAG CAT CTT CTA GCA GGC CTA CCA GTT
1506
Cys Phe Leu Asp Thr Ser Asn Gln His Leu Leu Ala Gly Leu Pro Val
455 460 465 470

CTC CGG CAT CTC AAC TTA AAA GGG AAT CAC TTT CAA GAT GGG ACT ATC
1554
Leu Arg His Leu Asn Leu Lys Gly Asn His Phe Gln Asp Gly Thr Ile
475 480 485

ACG AAG ACC AAC CTA CTT CAG ACC GTG GGC AGC TTG GAG GTT CTG ATT
1602
Thr Lys Thr Asn Leu Leu Gln Thr Val Gly Ser Leu Glu Val Leu Ile
490 495 500

TTG TCC TCT TGT GGT CTC CTC TCT ATA GAC CAG CAA GCA TTC CAC AGC
1650
Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp Gln Gln Ala Phe His Ser
505 510 515

TTG GGA AAA ATG AGC CAT GTA GAC TTA AGC CAC AAC AGC CTG ACA TGC
1698
Leu Gly Lys Met Ser His Val Asp Leu Ser His Asn Ser Leu Thr Cys
520 525 530

GAC AGC ATT GAT TCT CTT AGC CAT CTT AAG GGA ATC TAC CTC AAT CTG
1746
Asp Ser Ile Asp Ser Leu Ser His Leu Lys Gly Ile Tyr Leu Asn Leu
535 540 545 550

GCT GCC AAC AGC ATT AAC ATC ATC TCA CCC CGT CTC CTC CCT ATC TTG
1794
Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro Arg Leu Leu Pro Ile Leu
555 560 565

TCC CAG CAG AGC ACC ATT AAT TTA AGT CAT AAC CCC CTG GAC TGC ACT
1842
Ser Gln Gln Ser Thr Ile Asn Leu Ser His Asn Pro Leu Asp Cys Thr
570 575 580

TGC TCG AAT ATT CAT TTC TTA ACA TGG TAC AAA GAA AAC CTG CAC AAA
1890
Cys Ser Asn Ile His Phe Leu Thr Trp Tyr Lys Glu Asn Leu His Lys
585 590 595

CTT GAA GGC TCG GAG GAG ACA CGT GTG CAA AAC CCG CCA TCT CTA AGG
1938

```
Leu Glu Gly Ser Glu Glu Thr Arg Val Gln Asn Pro Pro Ser Leu Arg
    600                 605                 610

GGA GTT AAG CTA TCT GAT GTC AAG CTT TCC TGT GGG ATT ACA GCC ATA
1986
Gly Val Lys Leu Ser Asp Val Lys Leu Ser Cys Gly Ile Thr Ala Ile
615                 620                 625                 630

GGC ATT TTC TTT CTC ATA GTA TTT CTA TTA TTG TTG GCT ATT CTG CTA
2034
Gly Ile Phe Phe Leu Ile Val Phe Leu Leu Leu Leu Ala Ile Leu Leu
                635                 640                 645

TTT TTT GCA GTT AAA TAC CTT CTC AGG TGG AAA TAC CAA CAC TTT TAG
2082
Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp Lys Tyr Gln His Phe
            650                 655                 660

TGCTGAAGGT TTCCAGAGAA AGCAAATAAG TGTGCTTAGC AAAATTGCTC TAAGTGAAAG
2142

AACTGTCATC TGCTGGTGAC CAGACCAGAC TTTTCAGATT GCTTCCTGGA ACTGGGCAGG
2202

GACTCACTGT GCTTTTCTGA GCTTCTTACT CCTGTGAGTC CCAGAGCTAA AGAACCTTCT
2262

AGGCAAGTAC ACCGAATGAC TCAGTCCAGA GGGTCAGATG CTGCTGTGAG AGGCACAGAG
2322

CCCTTTCCGC ATGTGGAAGA GTGGGAGGAA GCAGAGGGAG GGACTGGGCA GGGACTGCCG
2382

GCCCCGGAGT CTCCCACAGG GAGGCCATTC CCCTTCTACT CACCGACATC CCTCCCAGCA
2442

CCACACACCC CGCCCCTGAA AGGAGATCAT CAGCCCCCAC AATTTGTCAG AGCTGAAGCC
2502

AGCCCACTAC CCACCCCCAC TACAGCATTG TGCTTGGGTC TGGGTTCTCA GTAATGTAGC
2562

CATTTGAGAA ACTTACTTGG GGACAAAGTC TCAATCCTTA TTTTAAATGA AAAAAGAAAA
2622

GAAAAGCATA ATA
2635
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 661 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Ala Phe Asp Val Ser Cys Phe Phe Trp Val Val Leu Phe Ser Ala
 1               5                  10                  15

Gly Cys Lys Val Ile Thr Ser Trp Asp Gln Met Cys Ile Glu Lys Glu
```

20 25 30

Ala Asn Lys Thr Tyr Asn Cys Glu Asn Leu Gly Leu Ser Glu Ile Pro
35 40 45

Asp Thr Leu Pro Asn Thr Thr Glu Phe Leu Glu Phe Ser Phe Asn Phe
50 55 60

Leu Pro Thr Ile His Asn Arg Thr Phe Ser Arg Leu Met Asn Leu Thr
65 70 75 80

Phe Leu Asp Leu Thr Arg Cys Gln Ile Asn Trp Ile His Glu Asp Thr
85 90 95

Phe Gln Ser His His Gln Leu Ser Thr Leu Val Leu Thr Gly Asn Pro
100 105 110

Leu Ile Phe Met Ala Glu Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys
115 120 125

His Leu Phe Leu Ile Gln Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro
130 135 140

Val His Asn Leu Glu Asn Leu Glu Ser Leu Tyr Leu Gly Ser Asn His
145 150 155 160

Ile Ser Ser Ile Lys Phe Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys
165 170 175

Val Leu Asp Phe Gln Asn Asn Ala Ile His Tyr Ile Ser Arg Glu Asp
180 185 190

Met Arg Ser Leu Glu Gln Ala Ile Asn Leu Ser Leu Asn Phe Asn Gly
195 200 205

Asn Asn Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe
210 215 220

Gln Ser Leu Asn Phe Gly Gly Thr Pro Asn Leu Ser Val Ile Phe Asn
225 230 235 240

Gly Leu Gln Asn Ser Thr Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu
245 250 255

Asp Ile Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys
260 265 270

Glu Met Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp
275 280 285

Ile Ser Ser Thr Thr Phe Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp
290 295 300

Leu Thr Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu
305 310 315 320

Asn Leu Leu Lys Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu
325 330 335

Cys Gln Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile
340 345 350

Arg Gly Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys
355 360 365

Leu Gly Asn Leu Gln Thr Leu Asp Leu Ser His Asn Asp Ile Glu Ala
370 375 380

Ser Asp Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr
385 390 395 400

Leu Asn Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe
405 410 415

Lys Glu Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu
420 425 430

His Ile Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln
435 440 445

Val Leu Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu
450 455 460

Leu Ala Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His
465 470 475 480

Phe Gln Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly
485 490 495

Ser Leu Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp
500 505 510

Gln Gln Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser
515 520 525

His Asn Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys
530 535 540

Gly Ile Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro
545 550 555 560

Arg Leu Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His
565 570 575

Asn Pro Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr
580 585 590

Lys Glu Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Arg Val Gln
595 600 605

Asn Pro Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser
610 615 620

Cys Gly Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu
625 630 635 640

Leu Leu Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp
645 650 655

Lys Tyr Gln His Phe
660

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

ACCATGG
7

WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid encoding a human BAS-1 protein or fragment thereof.

2. The isolated or recombinant nucleic acid of claim 1, wherein said nucleic acid comprises a sequence defined in SEQ ID NO: 1.

3. The nucleic acid of claim 2, wherein said nucleic acid exhibits at least about 80% identity to a natural cDNA encoding said segment.

4. The isolated or recombinant nucleic acid of claim 2 which encodes at least eight consecutive residues of SEQ ID NO: 2.

5. The nucleic acid of claim 4, which encodes at least twelve consecutive residues.

6. The nucleic acid of claim 4, wherein said nucleic acid exhibits at least about 80% identity to a natural cDNA encoding said segment.

7. A substantially pure BAS-1 protein or peptide thereof.

8. The protein or peptide of claim 7, selected from the group consisting of:
   a) a protein or peptide from a primate, including a human;
   b) a protein or peptide comprising at least one polypeptide segment defined in SEQ ID NO: 2;
   c) a protein or peptide which exhibits a post-translational modification pattern distinct from a natural BAS-1 protein; and
   d) a protein lacking the intracellular domain of BAS-1.

9. The protein or peptide of claim 8 comprising a segment exhibiting sequence homology to a corresponding portion of a human BAS-1 wherein:
   a) said homology is at least about 90% identity and said portion is at least about 9 amino acids;
   b) said homology is at least about 80% identity and said portion is at least about 17 amino acids; or
   c) said homology is at least about 70% identity and said portion is at least about 25 amino acids.

10. A fusion protein comprising a BAS-1 peptide of claim 7.

11. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.

12. An antibody or antibody binding fragment which specifically binds a recombinant or purified primate BAS-1 protein or fragment thereof.

13. The antibody of claim 12, wherein said BAS-1 protein is a primate protein, including one from a human.

14. The antibody of claim 12 wherein said antibody is raised against a peptide sequence defined in SEQ ID NO: 2.

15. The antibody of claim 12, wherein said antibody exhibits a Kd of at least about 10 μM.

16. The antibody of claim 12, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 12, wherein said antibody is labeled.

18. The antibody of claim 12, which:
   a) induces strong proliferation of B cells; and/or

67

b) protects B cells from irradiation-induced apoptosis.

19. An expression vector comprising the nucleic acid of claim 1.

20. A host cell comprising the vector of claim 19.

21. A method of screening a sample for a binding partner for BAS-1 comprising the steps of producing a purified or recombinant BAS-1 protein, and screening in said sample for a specific binding of said binding partner to said BAS-1 protein.

22. The method of claim 21, wherein said sample comprises proteins derived from a T cell, a dendritic cell, including a follicular dendritic cell, or a stromal cell, including a fibroblast cell, an endothelial cell, and an epithelial cell.

23. The method of claim 21, wherein said binding partner is an antibody, and said sample is a hybridoma supernatant.

24. A method of modulating physiology or development of a cell comprising contacting said cell with an agonist or antagonist of a BAS-1 protein.

25. The method of claim 24, wherein said antagonist is an antibody against a primate BAS-1 protein.

26. The method of claim 24, wherein said contacting is in combination with a mediator of a signal through the antigen receptor, the CD40:CD40 ligand pathway, or the CD28/CTLA-4:B7/B70 pathway.

This Sheet Has Been Replaced

9805673-2
IPOS
REPLACEMENT SHEET
17 NOV 1999

b) protects B cells from irradiation-induced apoptosis.

19. An expression vector comprising the nucleic acid of claim 1.

20. A host cell comprising the vector of claim 19.

21. A method of screening a sample for a binding partner for BAS-1 comprising the steps of producing a purified or recombinant BAS-1 protein, and screening in said sample for a specific binding of said binding partner to said BAS-1 protein.

22. The method of claim 21, wherein said sample comprises proteins derived from a T cell, a dendritic cell, including a follicular dendritic cell, or a stromal cell, including a fibroblast cell, an endothelial cell, and an epithelial cell.

23. The method of claim 21, wherein said binding partner is an antibody, and said sample is a hybridoma supernatant.

24. Use of an agonist or antagonist of a BAS-1 protein in contact with a cell for modulating physiology or development of said cell.

25. The use of claim 24, wherein said antagonist is an antibody against a primate BAS-1 protein.

26. The use of claim 24, wherein said contact is in combination with a mediator of a signal through the antigen receptor, the CD40:CD40 ligand pathway, or the CD28/CTLA-4:B7/B70 pathway.

9805673-2
IPOS

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/705, C12Q 1/68, C12N 15/62, C07K 19/00, C12N 5/10, C07K 16/28, G01N 33/68, 33/566, A61K 38/17, 39/395

A1

(11) International Publication Number: **WO 97/44452**

(43) International Publication Date: 27 November 1997 (27.11.97)

(21) International Application Number: PCT/US97/07648

(22) International Filing Date: 15 May 1997 (15.05.97)

(30) Priority Data:
08/649,553 17 May 1996 (17.05.96) US

(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

(72) Inventors: LIU, Yong-Jun; Pins 5, Résidence de Charrière Blanche, 24, chemin de Charrière Blanche, F-69130 Ecully (FR). FUGIER-VIVIER, Isabelle; 5, rue Mazenod, F-69003 Lyon (FR). BANCHEREAU, Jacques; 25, avenue Paul Santy, F-69130 Ecully (FR).

(74) Agents: POULKE, Cynthia, L. et al.; Schering-Plough Corporation, Patent Department K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HUMAN B-CELL ANTIGENS, RELATED REAGENTS

(57) Abstract

The purification and isolation of a gene which encodes a human B-cell antigen. Nucleic acids, proteins, antibodies, and other reagents useful in modulating development of cells, e.g., lymphoid, are provided, along with methods for their use.