IL-17A AND IL-17F ANTAGONISTS AND METHODS OF USING THE SAME

BACKGROUND OF THE INVENTION

[1] Cytokines are soluble, small proteins that mediate a variety of biological effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al., Annu. Rev. Biochem. 59*:783 (1990); Mosmann, *Curr. Opin. Immunol. 3*:311 (1991); Paul and Seder, *Cell 76*:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)).

[2] Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains.

[3] The demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists. For example, demonstrated *in vivo* activities of the pro-inflammatory cytokine family illustrates the enormous clinical potential of, and need for antagonists of pro-inflammatory molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

[4] Figures 1A and 1B are graphic representations of the exon structure of human IL-17RCx1 (SEQ ID NO:2). For those amino acid where codon was splied by exon/intron junction, the junction was moved to included the entire codon.

[5] Figures 2A and 2B are graphic representations of the exon structure of human IL-17RCx4 (SEQ ID NO:166).

[6] Figure 3 is a graphic representation of the exon structure of human IL-17RA (SEQ ID NO:21).

[7] Figures 4A and 4B are graphic representations of the exon structure of a preferred soluble polypeptide of the present invention as described herein and in SEQ ID NOs:157 and 158. This soluble polypeptide comprises exons from both human IL-17RA (SEQ ID NO:21) and human IL-17RCx1 (SEQ ID NO:2).

[8] Figure 5 is a graphical representation of a typical assay result using the protocol outlined in Example 34. The graph was generated using the Prizm software program. The Y values represent the MFI normalized to maximum and minimum (100% and 0%) based on ligand only and no ligand/no soluble receptor control wells, and thus the percent binding of the ligand to the cells. The software calculates the IC50 for each curve.

DETAILED DESCRIPTION OF THE INVENTION

[9] The present invention addresses these needs by providing antagonists to pro-inflammatory cytokines IL-17A and IL-17F. Specifically, the pro-inflammatory cytokines IL-17A and IL-17F have a high degree of sequence similarity, share many biological properties, and are both produced by activated T cells. They have both been implicated as factors that contribute to the progression of various autoimmune and inflammatory diseases including rheumatoid arthritis and asthma. In fact, reagents that negate IL-17A function significantly ameliorate disease incidence and severity in several mouse models of human disease. IL-17A mediates its effects through interaction with its cognate receptor, the IL-17 receptor (IL-17R), but the receptor for IL-17F had not yet been identified. Previously, we had reported that IL-17RC is a receptor for both IL-17A and IL-17F, and binds both with a similar high affinity. IL-17R on the other hand, binds IL-17A with high affinity, but binds IL-17F with very low affinity. Consistent with this, it has been shown that a soluble form of IL-17R blocks IL-17A binding and signaling in cells expressing either receptor, but does not interfere with binding or function of IL-17F to IL-17RC.

[10] Since IL-17A intervention has been proposed as an effective therapy for several auto-immune diseases, using the antagonists of the present invention, which may block, inhibit, reduce, antagonize or neutralize the activity of IL-17A, IL-17F, or both IL-17A and IL-17F, which include soluble IL-17RC and IL-17RC/IL-17RA receptors, will have advantages over therapies that target only one of these two cytokines. The invention further provides uses therefor in inflammatory disease, as well as related compositions and methods.

A) Overview

[11] Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

[12] Though the genesis of these diseases often involves multi-step pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these

pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

[13] Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases (such as rheumatoid arthritis, immune mediated renal disease, hepatobiliary diseases, inflammatory bowel disease (IBD), psoriasis, and asthma), non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

[14] T lymphocytes (T cells) are an important component of a mammalian immune response. T cells recognize antigens which are associated with a self-molecule encoded by genes within the major histocompatibility complex (MHC). The antigen may be displayed together with MHC molecules on the surface of antigen presenting cells, virus infected cells, cancer cells, grafts, etc. The T cell system eliminates these altered cells which pose a health threat to the host mammal. T cells include helper T cells and cytotoxic T cells. Helper T cells proliferate extensively following recognition of an antigen-MHC complex on an antigen presenting cell. Helper T cells also secrete a variety of cytokines, i.e., lymphokines, which play a central role in the activation of B cells, cytotoxic T cells and a variety of other cells which participate in the immune response.

[15] A central event in both humoral and cell mediated immune responses is the activation and clonal expansion of helper T cells. Helper T cell activation is initiated by the interaction of the T cell receptor (TCR)--CD3 complex with an antigen-MHC on the surface of an antigen presenting cell. This interaction mediates a cascade of biochemical events that induce the resting helper T cell to enter a cell cycle (the G0 to G1 transition) and results in the expression of a high affinity receptor for IL-2 and sometimes IL-4. The activated T cell progresses through the cycle proliferating and differentiating into memory cells or effector cells.

[16] In addition to the signals mediated through the TCR, activation of T cells involves additional costimulation induced by cytokines released by the antigen presenting cell or through interactions with membrane bound molecules on the antigen presenting cell and the T cell. The cytokines IL-1 and IL-6 have been shown to provide a costimulatory signal. Also, the interaction between the B7 molecule expressed on the surface of an antigen presenting cell and CD28 and CTLA-4 molecules expressed on the T cell surface effect T cell activation. Activated T cells express an increased number of cellular adhesion molecules, such as ICAM-1, integrins, VLA-4, LFA-1, CD56, etc.

[17] T-cell proliferation in a mixed lymphocyte culture or mixed lymphocyte reaction (MLR) is an established indication of the ability of a compound to stimulate the immune system. In many immune responses, inflammatory cells infiltrate the site of injury or infection. The migrating cells may be neutrophilic, eosinophilic, monocytic or lymphocytic as can be determined by histologic

examination of the affected tissues. Current Protocols in Immunology, ed. John E. Coligan, 1994, John Wiley & Sons, Inc.

[18] Immune related diseases could be treated by suppressing the immune response. Using soluble receptors and/or neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

[19] Interleukin-17 (IL-17A) has been identified as a cellular ortholog of a protein encoded by the T lymphotropic Herpes virus Saimiri (HSV) [see, Rouvier et al., J. Immunol., 150(12): 5445-5456 (19993); Yao et al., J. Immunol., 122(12):5483-5486 (1995) and Yao et al., Immunity, 3(6):811-821 (1995)]. Subsequent characterization has shown that this protein is a potent cytokine that acts to induce proinflammatory responses in a wide variety of peripheral tissues. IL-17A is a disulfide-linked homodimeric cytokine of about 32 kDa which is synthesized and secreted only by CD4+activated memory T cells (reviewed in Fossiez et al., Int. Rev. Immunol., 16: 541-551 [1998]). Specifically, IL-17 is synthesized as a precursor polypeptide of 155 amino acids with an N-terminal signal sequence of 19-23 residues and is secreted as a disulfide-linked homodimeric glycoprotein. Il-17A is disclosed in WO9518826 (1995), WO9715320 (1997) and WO9704097 (1997), as well as US Patent No. 6,063,372.

[20] Despite its restricted tissue distribution, IL-17A exhibits pleitropic biological activities on various types of cells. IL-17A has been found to stimulate the production of many cytokines. It induces the secretion of IL-6, IL-8, IL-12, leukemia inhibitory factor (LIF), prostaglandin E2, MCP-1 and G-CSF by adherent cells like fibroblasts, keratinocytes, epithelial and endothelial cells. IL-17A also has the ability to induce ICAM-1 surface expression, proliferation of T cells, and growth and differentiation of CD34.sup.+ human progenitors into neutrophils. IL-17A has also been implicated in bone metabolism, and has been suggested to play an important role in pathological conditions characterized by the presence of activated T cells and TNF-.alpha. production such as rheumatoid arthritis and loosening of bone implants (Van Bezooijen et al., J. Bone Miner. Res. 14: 1513-1521 [1999]). Activated T cells of synovial tissue derived from rheumatoid arthritis patients were found to secrete higher amounts of IL-17A than those derived from normal individuals or osteoarthritis patients (Chabaud et al., Arthritis Rheum. 42: 963-970 [1999]). It was suggested that this proinflammatory cytokine actively contributes to synovial inflammation in rheumatoid arthritis. Apart from its proinflammatory role, IL-17A seems to contribute to the pathology of rheumatoid arthritis by yet another mechanism. For example, IL-17A has been shown to induce the expression of osteoclast differentiation factor (ODF) mRNA in osteoblasts (Kotake et al., J. Clin. Invest., 103: 1345-1352 [1999]). ODF stimulates differentiation of progenitor cells into osteoclasts, the cells involved in bone resorption.

[21] Since the level of IL-17A is significantly increased in synovial fluid of rheumatoid arthritis patients, it appears that IL-17A induced osteoclast formation plays a crucial role in bone resorption in rheumatoid arthritis. IL-17A is also believed to play a key role in certain other autoimmune disorders such as multiple sclerosis (Matusevicius et al., Mult. Scler., 5: 101-104 [1999]). IL-17A has further been shown, by intracellular signalling, to stimulate Ca.sup.2+ influx and a reduction in [cAMP], in human macrophages (Jovanovic et al., J. Immunol., 160:3513 [1998]). Fibroblasts treated with IL-17A induce the activation of NF-.kappa.B, [Yao et al., Immunity, 3:811 (1995), Jovanovic et al., supra], while macrophages treated with it activate NF-.kappa.B and mitogen-activated protein kinases (Shalom-Barek et al., J. Biol. Chem., 273:27467 [1998]).

[22] Additionally, IL-17A also shares sequence similarity with mammalian cytokine-like factor 7 that is involved in bone and cartilage growth. Other proteins with which IL-17A polypeptides share sequence similarity are human embryo-derived interleukin-related factor (EDIRF) and interleukin-20.

[23] Consistent with IL-17A's wide-range of effects, the cell surface receptor for IL-17A has been found to be widely expressed in many tissues and cell types (Yao et al., Cytokine, 9:794 [1997]). While the amino acid sequence of the human IL-17A receptor (IL-17R) (866 amino acids) predicts a protein with a single transmembrane domain and a long, 525 amino acid intracellular domain, the receptor sequence is unique and is not similar to that of any of the receptors from the cytokine/growth factor receptor family. This coupled with the lack of similarity of IL-17A itself to other known proteins indicates that IL-17A and its receptor may be part of a novel family of signalling proteins and receptors. It has been demonstrated that IL-17A activity is mediated through binding to its unique cell surface receptor, wherein previous studies have shown that contacting T cells with a soluble form of the IL-17A receptor polypeptide inhibited T cell proliferation and IL-2 production induced by PHA, concanavalin A and anti-TCR monoclonal antibody (Yao et al., J. Immunol., 155:5483-5486 [1995]). As such, there is significant interest in identifying and characterizing novel polypeptides having homology to the known cytokine receptors, specifically IL-17A receptors.

[24] The expression pattern of IL-17F appears to be similar to that of IL-17A, such that it includes only activated CD4+ T cells and monocytes (Starnes et al. J. Immunol. 167: 4137-4140 [2001]). IL-17F has been demonstrated to induce G-CSF, IL-6, and IL-8 in fibroblasts (Hymowitz et al, EMBO J. 20:5322-5341 [2001]) and TGF-b in endothelial cells (Starnes et al. J. Immunol. 167: 4137-4140 [2001]). It has recently been reported that IL-23, a cytokine produced by dendritic cell, can mediate the production of both IL-17A and IL-17F, primarily in memory T cells (Aggarwal et al. J. Biol. Chem. 278:1910-1914 [2003]).

[25] Moreover, over expression or upregulation of both IL-17A and IL-17F have been shown in arthritic and asthmatic individuals (reviewed in Moseley et al. CytokineGrowth Factor Rev

14:155-174 [2003]). With regards to arthritis, these cytokines act in a manner characteristic to the cartilage and joint destruction that is associated with rheumatoid- and osteo-arthritis. For example, IL-17A and IL-17F have been demonstrated to enhance matrix degradation in articular cartilage explants via release of cartilage proteoglycan glycosaminoglycans and collagen fragments, while inhibiting the synthesis of new proteoglycans and collagens (Cai et al. Cytokine 16:10-21 [2001]; Attur et al Arthritis Rheum 44:2078-2083 [2001]).

[26] Similar to IL-17A, overexpression of IL-17F in mice has also been shown to increase lung neutrophil recruitment and result in increased expression of Th1-associated cytokines in the lung, including IL-6, IFN-gamma, IP-10 and MIG (Starnes et al. J. Immunol. 167: 4137-4140 [2001]). IL-17F was also upregulated in T cells from allergen-challenged asthmatics (Kawaguchi et al J. Immunol 167:4430-4435 [2001]), and found to induce IL-6 and IL-8 production in NHBE. In contrast to IL-17A, IL-17F appears to inhibit angiogenesis in vitro (Starnes et al. J. Immunol. 167: 4137-4140 [2001]).

[27] IL-17F mRNA was not detected by northern blot in various human tissues but was dramatically induced upon activation of CD4+ T cells and monocytes. Id. In mice, Th2 cells and mastr cells were found to express IL-17F upon activation. See Dumont, Expert Opin. Ther. Patents 13(3) (2003). Like IL-17A, the expression of IL-17F was alos found to be upregulated by IL-23 in mouse.

[28] The Il-17 cytokine/receptor families appear to represent a unique signaling system within the cytokine network that will offer innovative approaches to the manipulation of immune and inflammatory responses. Accordingly, the present invention is based on the discovery of a new IL-17 family receptor, IL-17RC and its ability to bind both IL-17A and IL-17F.

[29] IL-17RC was initially identified using a bioinformatics approach to search for proteins related to IL-17RA and identified through a cDNA encoding the IL-17 receptor-related protein IL-17RC. In spite of its obvious similarity to the IL-17 receptor (IL-17RA), which binds to the prototypical member of the IL-17 family IL-17A, and the identification of five other members of the IL-17 cytokine family, a specific ligand for IL-17RC had not been previously reported. However, IL-17A and IL-17F were identified as the specific ligands for IL-17RC as described in US Patent Application No. 11/150,533, filed on June 10, 2005 and published as US Patent Publication No. 20060002925. Specifically, these ligands were identified using Baby Hamster Kidney cells (BHK) that were stably transfected with constructs encoding either human IL-17RA (hIL-17RA) or IL-17RC (hIL-17RC). Expression of receptors on the surface was confirmed by FACS analysis using either a monoclonal antibody to hIL-17RA or a polyclonal antiserum to hIL-17RC. To assess cytokine binding, biotinylated forms of human IL-17A, C, D, E, and F and fluorochrome-conjugated streptavidin were used to detect cytokine binding to transfected cells by flow cytometry. The results clearly showed that stably transfected BHK cells expressing hIL-17RA clearly bound human IL-17A

(hIL-17A) as expected, whereas those transfected with empty expression vector failed to bind any members of the IL-17 family tested. Relatively weak binding of human IL-17F (hIL-17F) to hIL-17RA-transfected cells was also observed, but there was no significant binding of other members of the IL-17 family tested. Other IL-17 family members were examined for binding of to hIL-17RC-transfected cells and it was noted that these cells showed significant binding to hIL-17F. In addition, significant binding of hIL-17A to these cells was seen, but no binding of hIL-17C, D, or E. This data proved that hIL-17RC was the receptor for both hIL-17F and hIL-17A.

[30] Additionally, the level of fluorescence over a range of cytokine concentrations was examined to determine relative affinities of hIL-17A and F for hIL-17RA and hIL-17RC. By comparing mean fluorescence intensities of the individual cytokines on each transfectant, it was noted that hIL-17A bound much better to hIL-17RA than hIL-17F did, but that both cytokines seemed to bind equally well to hIL-17RC-transfected cells. Interestingly, cytokine binding to cells that expressed both receptors seemed to be additive, with no evidence of cooperativity.

[31] Next, the specificity of this binding was investigated by attempting to compete for binding with unlabeled cytokine. Transfected BHK cells were incubated with a fixed concentration of biotinylated cytokine and increasing concentrations of unlabeled cytokine and the amount of bound biotinylated material was quantitated by FACS. It was shown that the binding of both hIL-17A and F to hIL-17RC was specific since increasing concentrations of unlabeled cytokine interfered with binding of the biotinylated material. In fact, unlabeled hIL-17A and F effectively cross-competed for binding of biotinylated forms of both cytokines to hIL-17RC-transfected cells, suggesting that the two cytokines were binding hIL-17RC with similar affinities, and that they were binding to overlapping, if not identical sites. Uunlabeled hIL-17A also effectively competed for binding of both biotinylated hIL-17A and F to hIL-17RA-transfected cells, while unlabeled hIL-17F showed essentially no ability to compete for hIL-17A binding to hIL-17RA. This indicated that although hIL-17F showed specific binding to hIL-17RA, the avidity of this interaction appeared to be significantly lower than the interaction of hIL-17A and hIL-17RA.

[32] Saturation binding studies were done to measure the affinity of hIL-17A and F binding to hIL-17RC and hIL-17RA. BHK cell lines stably expressing hIL-17RA or hIL-17RC were incubated with iodinated hIL-17A or F under saturation binding conditions to determine the affinity constants of each cytokine for each receptor. hIL-17A bound both hIL-17RA and hIL-17RC with comparable affinities (Table 1). Specifically, BHK cells transfected with the indicated receptor were used to establish K_d values for hIL-17 A and hIL-17F as described in Methods. Results shown are mean K_d values derived from triplicate determinations.

Table 1

	hIL-17A	hIL-17F

hIL-17RC (x1)[1]	0.6 nM	1.0 nM
hIL-17RA	1.9 nM	1.5 □M

[1]Denotes the x1 splice variant of hIL-17RC.

[33] In addition, the affinity of hIL-17F for hIL-17RC was very similar to the affinity of hIL-17A for this receptor (see Table 1 above). However, consistent with results obtained using biotinylated cytokines, the affinity of hIL-17F for hIL-17RA was roughly 1000-fold lower relative to other affinities measured (*Id.*). This indicates that hIL-17A and F bind hIL-17RC with similar affinities, but their affinities for hIL-17RA differ dramatically.

[34] The observation that hIL-17RC bound both hIL-17A and F with high affinity suggests that cells expressing hIL-17RC should be equally capable of responding to hIL-17A and F. On the other hand, since hIL-17RA bound hIL-17A with high affinity, but hIL-17F about 1000-fold less well, the implication is that cells expressing hIL-17RA would, under physiologic conditions, only respond to hIL-17A. Previously, it had been shown that hIL-17RA is expressed ubiquitously, but its expression has been reported to be higher in hematopoietic cells with lower expression in other tissues. Therefore, the expression of hIL-17RC was examined to determine the extent of overlap in the expression patterns. Northern blot analysis showed that hIL-17RC was expressed at high levels in glandular tissues such as adrenal gland, prostate, liver, and thyroid with no detectable expression in hematopoietic tissues.

[35] To further investigate expression of these receptors in hematopoietic cells, the binding of biotinylated hIL-17A and F to peripheral blood mononuclear cells (PBMC) by multiparameter FACS analysis was also examined. Results indicated that hIL-17A bound to virtually all PBMC subsets examined, whereas hIL-17F failed to show detectable binding to any of these populations. This is consistent with the capacity of hIL-17RA to bind hIL-17A with high affinity, but not hIL-17F, and with the to detect hIL-17RC mRNA in PBMC. Collectively, these data indicate that IL-17RC is preferentially expressed in non-hematopoietic tissues, while IL-17RA is preferentially expressed in hematopoietic cells.

[36] The high affinity binding of hIL-17A and F to hIL-17RC-transfected cells suggests that an efficiaous therapeutic might be a soluble form of hIL-17RC. Such a molecule would be an effective antagonist of these two cytokines. To test this directly, a soluble form of human hIL-17RC was produced as an Fc-fusion protein and tested its ability to inhibit the binding of both hIL-17A and F. These effects were then compared with results obtained using a soluble form of hIL-17RA. Increasing concentrations of hIL-17RC-Ig or hIL-17RA-Ig were included in binding reactions and FACS analysis was used to assess effects of the soluble receptors on binding of biotinylated cytokines to stably transfected BHK cells. Soluble hIL-17RC inhibited the binding of both hIL-17A and F to a

similar extent, whereas an Fc-fusion protein of another member of the IL-17R family, hIL-17RD, had no effect. On the other hand, soluble hIL-17RA effectively blocked binding of hIL-17A, but had essentially no effect on the binding of hIL-17F. Similar results were obtained examining binding of hIL-17A to hematopoietic cells. This binding was effectively blocked using hIL-17RA-Ig and hIL-17RC-Ig, but not hIL-17RD-Ig. These data are consistent with results obtained from affinity measurements and indicate that the soluble receptors are behaving the same as their membrane-anchored forms.

[37] As an additional assessment of the capacity of the human hIL-17RC-Ig to bind to hIL-17A and F, the affinity of the soluble receptor for these cytokines was assessed using Biacore analysis. Soluble hIL-17RC bound to both hIL-17A and F with high affinity (Table 2), providing additional support for the idea of using this reagent as an antagonist for the effects of both hIL-17A and F in vivo. Specifically, soluble receptors were captured onto chips and binding experiments were performed as described below. ND = no detectable binding.

Table 2

hIL-17RC-Ig	k_a (on-rate)	k_d (off-rate)	K_D
mIL17A		ND	
mIL17F		ND	
hIL17A	1.05E+06	4.90E-04	0.469nM
	1.24E+06	4.38E-04	0.352nM
hIL17F	9.91E+05	4.31E-04	0.435nM
	1.11E+06	3.84E-04	0.346nM

mL-17RA-Ig	k_a (on-rate)	k_d (off-rate)	K_D
mIL17A	9.78E+05	6.79E-05	0.069nM
	1.12E+06	7.99E-05	0.072nM
mIL17F		ND	

[38] The number of splice variants in humans is much greater and therefore we performed our initial experiments on only a subset of these molecules. Those chosen for this analysis also differed in their inclusion or exclusion of exon 7, but, unlike the mouse, all splice variants incorporated all of exon 8. The cryptic splice acceptor found in the middle of the mouse exon 8 sequence is not present in human exon 8. However, the other splice variants tested either included or excluded hIL-17RC exon 12. These variants were designated hIL-17RCx1 (identical in exon composition to mouse x1 above), hIL-17RCx4 (identical in exon composition to mouse x4 above), hIL-17RCx2, and hIL-17RCx7. Again, these splice variants were transiently expressed in 293F cells and were tested for their ability to bind biotinylated mouse and human IL-17A and F and the results are summarized in Table 3.

Table 3

		Exons[1]			**Cytokine Binding[2]**			
	Variant	7	8	12	hIL-17A	hIL-17F	mIL-17A	mIL-17F
Human	IL-17RCx4	+	+	+	+	+	-	+
	IL-17RCx1	-	+	+	+	+	-	-
	IL-17RCx2	-	+	-	-	-	-	-
	IL-17RCx7	+	+	-	-	-	-	-

[1]Denotes exons completely included in transcript. [2](+) indicates a detectable, significant cytokine binding as assessed by a significant increase in fluorescence by FACS. (-) indicates no significant change in fluorescence.

[39] Consistent with the experiments presented earlier, hIL-17RCx1 bound to both hIL-17A and F, but did not bind to either mouse cytokine. hIL-17RCx4 also bound to both human cytokines, and like its mouse counterpart, it bound to mIL-17F, but not mIL-17A. hIL-17RCx2 and x7 failed to bind any of the four cytokines tested, although they were clearly expressed on the surface of transfected cells since a polyclonal antiserum against hIL-17RC stained $CD8^+$ cells (data not shown). These binding results were faithfully recapitulated in stably transfected BHK cells as well. Collectively, these data support conclusions regarding essential portions of the IL-17RC protein required for binding to the human cytokines.

[40] Numerous publications have implicated IL-17A and, to a lesser extent, IL-17F as contributing to disease progression and severity in mouse collagen-induced arthritis (CIA) and human rheumatoid arthritis. The expression of both mIL-17A and F in the joints or draining lymph nodes (DLN) from mice that had been immunized with collagen to induce CIA was examined. Analysis by real-time PCR clearly demonstrated that both cytokines were upregulated in both tissues in diseased mice relative to unimmunized controls, clearly indicating that expression correlated with disease. In addition, the relative expression of mIL-17RA and mIL-17RC was also examined in the same tissues. However, in this case, there was not a reproducible correlation of expression of either receptor with disease. Moreover, what was obvious was the discrepancy in expression comparing DLN to non-hematopoietic tissue (hind foot). Consistent with the previous results looking at expression of the human receptors, mIL-17RA was found to be more highly expressed in hematopoietic tissue, and mIL-17RC to be more highly expressed in non-hematopoietic tissue. This data suggests that expression of mIL-17A and mIL-17F expression correlates with disease, that both of the requisite receptors are present in diseased and normal tissue, and suggests that neutralization of these cytokines may be an effective therapy to prevent disease progression.

[41] Accordingly, the cognate receptor for IL-17A and F has been shown to be IL-17RC. Notably, hIL-17RC binds to hIL-17A and F with similar affinities. Since these two members of the IL-17 family share 55% sequence identity, it is perhaps not surprising that they share receptors. However, hIL-17RA binds hIL-17A with high affinity, but binds hIL-17F with an affinity that is nearly 1000-fold lower, suggesting that under physiologic conditions, hIL-17RA would not bind hIL-17F. The implication is that cells that express hIL-17RC should respond to both hIL-17A and F, whereas cells that only express hIL-17RA will only respond to IL-17A. This difference has the potential to impact how these cytokines affect different tissues. Through expression analysis it was shown that although IL-17RA is expressed ubiquitously, it is more highly expressed in hematopoietic cells, whereas IL-17RC tends to be expressed in non-hematopoietic tissues with no expression in

hematopoietic cells. Consistent with this, all subsets of human peripheral blood mononuclear cells bind hIL-17A, but do not bind hIL-17F. Moreover, this suggests that non-hematopoietic tissues should respond to both IL-17A and F, whereas hematopoietic cells should only respond to IL-17A.

[42] This examination of cytokine binding to the different IL-17RC splice variants has revealed two portions of the receptor that are essential for cytokine binding, and there are subtle differences in the binding characteristics of the mouse and human cytokines. Moreover, these characteristics are consistent for the cytokines regardless of the species of the receptor examined. As shown from the data presented in Table 3, exon 12 and all of exon 8 are required for hIL-17A and F to bind to IL-17RC, since these cytokines only bind to the human x1 variants and the human x4 variants. Each of these isoforms includes all of exon 8 and exon 12, although they differ with respect to whether exon 7 is included or not. This implies that exon 7 is dispensable for binding of the human cytokines.

[43] The importance of generating an antagonist to both IL-17A and IL-17F function seems clear from available information that shows a strong correlation between IL-17A and F expression and progression of a number of autoimmune and inflammatory diseases. These two cytokines induce other inflammatory cytokines and chemokines as well as matrix metalloproteases, which contribute to collagen and bone destruction in autoimmune arthritis. This reagent should serve as an effective therapeutic for rheumatoid arthritis and in other inflammatory diseases in which hL-17A and F play a role.

[44] Thus, soluble forms of human IL-17RC were developed to serve as an antagonist to both IL-17A and IL-17F. Therapeutically, these soluble IL-17RC polypeptides were efficacious. However, due to numerous factors, soluble IL-17RC is not easily secreted from the numerous and varying production systems available in the art. Nor is it secreted in adequate quantities needed for manufacturing purposes. Thus, there is a need in the art to develop antagonists to IL-17A and IL-17F that can be expressed and secreted in quantities that can be scaled up for manufacturing.

[45] Accordingly, the present invention answers this need by providing IL-17A and IL-17F antagonists that can be expressed and secreted. Specifically, the present invention is based on the development and discovery of a number non-naturally occurring soluble molecules or soluble polypeptides that bind to, antagonize and/or block the binding of IL-17A and IL-17F to their cognate receptor(s). These soluble polypeptides comprise portions of IL-17RC. These soluble polypeptides can also comprise portions of both IL-17RC and IL-17RA ("IL-17RC/IL-17RA").

[46] One such preferred embodiment is described in Figures 4A and 4B, as well as in SEQ ID NOs:157 and 158. This soluble polypeptide comprises exons 1-6 of human IL-17RA (SEQ ID NO:21) and exons 8-16 of human IL-17RCx1 (SEQ ID NO:2). More specifically, this soluble polypeptide is fused if an Fc molecule, such as Fc5 as contained in SEQ ID Nos:157 and 158.

However, one skilled in the art would easily recognize that any Fc molecule can be utilized as well as any other molecule that would result in dimerization.

[47] As such, antagonists to IL-17F and IL-17A activity, such as IL-17RC and IL-17RC/IL-17RA soluble receptors of the present invention, are useful in therapeutic treatment of inflammatory diseases, particularly as antagonists to both IL-17F and IL-17A singly or together in the treatment of diseases involving these molecules. Moreover, antagonists to IL-17A and IL-17F activity, such as the soluble receptors of the present invention, are useful in therapeutic treatment of other inflammatory diseases for example as bind, block, inhibit, reduce, antagonize or neutralize IL-17F and IL-17A (either individually or together) in the treatment of psoriasis, atopic and contact dermatitis, IBD, IBS, colitis, endotoxemia, arthritis, rheumatoid arthritis, psoriatic arthritis, adult respiratory disease (ARD), septic shock, multiple organ failure, inflammatory lung injury such as asthma, chronic obstructive pulmonary disease (COPD), airway hyper-responsiveness, chronic bronchitis, allergic asthma, bacterial pneumonia, psoriasis, eczema, , and inflammatory bowel disease such as ulcerative colitis and Crohn's disease, *helicobacter pylori* infection. intraabdominal adhesions and/or abscesses as results of peritoneal inflammation (i.e. from infection, injury, etc.), systemic lupus erythematosus (SLE), multiple sclerosis, systemic sclerosis, nephrotic syndrome, organ allograft rejection, graft vs. host disease (GVHD), kidney, lung, heart, etc. transplant rejection, streptococcal cell wall (SCW)-induced arthritis, osteoarthritis, gingivitis/periodontitis, herpetic stromal keratitis, cancers including prostate, renal, colon, ovarian, cervical, leukemia, angiogenesis, restenosis and kawasaki disease.

[48] Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors include monomers, homodimers (e.g., PDGF receptor $\alpha\alpha$ and $\beta\beta$ isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor $\alpha\beta$ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures and functions. Class I hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif. Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors. Cytokine receptor structure has been reviewed by Urdal, Ann. Reports Med. Chem. 26:221-228, 1991 and Cosman, Cytokine 5:95-106, 1993. It is generally believed that under selective pressure for organisms to acquire new

biological functions, new receptor family members arose from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members.

[49] Accordingly, the present invention is directed to Il-17A and IL-17F antagonists that block each respective ligand from binding and/or signaling through its corresponding receptor or receptors.

[50] In preferred embodiments, such antagonists are based on IL-17RC's polypeptide structure as depicted in Figures 1-4. The IL-17RC receptor has a large number of splice variants based on the inclusion or exclusion of specific exons. As described below, some of these exons are required for ligand (IL-17A and/or IL-17F) binding.

[51] The present invention is based in part of the discovery of structural similarity ("domains") between IL-17RC and other members of the IL-17 family, such as IL-17RA (SEQ ID NO:21). Specifically, three domains were identified:

1) Domain 1 (SEQ ID NOs: 159 and 160) comprises exons 8-10 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 193-276 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 208-291 of (SEQ ID NO:166).

2) Domain 2 (SEQ ID NOs: 161 and 162) comprises exons 11-13 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 277-370 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 292-385 of (SEQ ID NO:166).

3) Domain 3 (SEQ ID NOs: 163 and 164) comprises exons 8-10 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 371-447 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 386-462 of (SEQ ID NO:166).

[52] Thus, the present invention is directed to soluble IL-17RC polypeptides based on different combinations of the exons depicted in Figure 1. Specifcally, examples of these soluble polypeptides include:

1) Variant 1210 (SEQ ID NOs: 67 and 68) which includes exons 1-6 and 8-16 of human IL-17RCx1, fused to Fc10 (SEQ ID NOs: 174 and 175) via a linker (SEQ ID NOs: 176 and 177). Variant 1210 also has a pre-pro signal peptide from otPA (polypeptide sequence shown in SEQ ID NO: 178). Fc5, or any equivalent known in the art, may also be used in place of Fc10.

2) Variant 1390 (SEQ ID NOs: 69 and 70) which includes exons 1-6 and 8-16 of human IL-17RCx1, fused to Fc10 (SEQ ID NOs: 174 and 175) . Variant 1390 also has the native signal sequence. Fc5, or any equivalent known in the art, may also be used in place of Fc10.

3) Variant 1341 (SEQ ID NOs: 71 and 72) which includes exons 1-6 of murine IL-17RA and 8-16 of human IL-17RCx1, fused to Fc10 (SEQ ID NOs: 174 and 175) via a linker (SEQ ID NOs: 176

and 177) . Variant 1341 also has a signal peptide from murine IL-17RA (SEQ ID NO:181). Fc5, or any equivalent known in the art, may also be used in place of Fc10.

4) Variant 1342 (SEQ ID NOs: 73 and 74) which includes exons 8-16 of human IL-17RCx1, fused to Fc10 (SEQ ID NOs: 174 and 175) via a linker (SEQ ID NOs: 176 and 177). Variant 1342 also has a pre-pro signal peptide from otPA (polypeptide sequence shown in SEQ ID NO: 178). Fc5, or any equivalent known in the art, may also be used in place of Fc10.

5) Variant S1 (SEQ ID NOs: 77 and 78) which includes exons 1-7 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S1 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

6) Variant S2 (SEQ ID NOs: 81 and 82) which includes exons 1-8 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S2 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

7) Variant S3 (SEQ ID NOs: 85 and 86) which includes exons 1-9 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S3 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

8) Variant S4 (SEQ ID NOs: 89 and 90) which includes exons 1-10 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S4 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

9) Variant S5 (SEQ ID NOs: 93 and 94) which includes exons 1-11 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S5 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

10) Variant S6 (SEQ ID NOs: 97 and 98) which includes exons 14-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S6 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

11) Variant S7 (SEQ ID NOs: 101 and 102) which includes exons 11-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S7 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

12) Variant S10 (SEQ ID NOs: 105 and 106) which includes exons 7-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S10 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

13) Variant S11 (SEQ ID NOs: 109 and 110) which includes exons 1-7 and 14-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S11 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

14) Variant S12 (SEQ ID NOs: 113 and 114) which includes exons 1-7 and 11-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S12 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

15) Variant S13 (SEQ ID NOs: 117 and 118) which includes exons 1-13 of human IL-17RCx1 and exons 7-9 of human IL-17RA, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S13 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

16) Variant S14 (SEQ ID NOs: 121 and 122) which includes exons 1-6 of murine IL-17RA, exons 8-13 of human IL-17RCx1 and exons 7-9 of murine IL-17RA, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant S13 also has the native signal sequence. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

17) Variant 1407 (SEQ ID NOs: 139 and 140) which includes exons 1-10 of human IL-17RA and 8-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant 1407 also has the native signal peptide from human IL-17RC. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

18) Variant 1459 (SEQ ID NOs: 151 and 152) which includes exons 1-6 and 8-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180) with a Leu21Ala substitution (as compared with IL-17RCx1). Variant 1459 also has a pre-pro signal peptide from otPA (polypeptide sequence shown in SEQ ID NO: 178). Fc10, or any equivalent known in the art, may also be used in place of Fc5.

19) Variant 1454 (SEQ ID NOs: 157 and 158) which includes exons 1-6 of human IL-17RA and 8-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant 1454 also has the native signal peptide from human IL-17RA. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

[53] The above-described variants represent only a limited number of the embodiments of the present invention. One skilled in the art could readily, and without undue experimentation, design and test other IL-17RC and/or IL-17RC/IL-17RA variants based on the teachings of the present application and in particular Figures 1-4 included herewith. For instance, other signal peptides which may be used in place of those disclosed above include: human growth hormone signal peptide (SEQ ID NOs: 168 and 169), murine immunoglobulin heavy chain variable region (VH 26-10) (SEQ ID NOs: 170 and 171), or human CD33 (SEQ ID NOs: 172 and 173).

[54] Amongst other inventions, the present invention provides novel uses for the soluble receptors of the present invention. These soluble receptors can be based solely on IL-17RC (designated "IL-17RC" or "soluble IL-17RC" or "sIL-17RC", all of which may be used herein interchangeably), or can be based on combining portions of IL-17RA with IL-17RC ("IL-17RC/IL-17RA" or "hybrid RC/RA" "RC/RA" or any variation thereof", all of which may be used herein interchangeably). The present invention also provides soluble IL-17RC and IL-17RC/IL-17RA polypeptide fragments and fusion proteins, for use in human inflammatory and autoimmune diseases. The soluble receptors of the present invention can be used to block, inhibit, reduce, antagonize or

neutralize the activity of either IL-17F or IL-17A, or both IL-17A and IL-17F in the treatment of inflammation and inflammatory dieases such as psoriasis, psoriatic arthritis, rheumatoid arthritis, endotoxemia, IBD, IBS, colitis, asthma, allograft rejection, immune mediated renal diseases, hepatobiliary diseases, multiple sclerosis, atherosclerosis, promotion of tumor growth, or degenerative joint disease and other inflammatory conditions disclosed herein.

[55] An illustrative nucleotide sequence that encodes human IL-17RC ("IL-17RCx1) is provided by SEQ ID NO:1; the encoded polypeptide is shown in SEQ ID NO:2. IL-17RC functions as a receptor for both IL-17A (SEQ ID NOS:13 & 14) and IL-17F (SEQ ID NOS:15 & 16). IL-17RC can act as a monomer, a homodimer or a heterodimer. Preferably, IL-17RC acts as a homodimeric receptor for both IL-17A and/or IL-17F. As described in the present application, either the monomeric or the homodimeric receptor can comprise IL-17RC alone, or it may comprise portions of other IL-17 family receptors, such as IL-17RA (IL-17RC/IL-17RA"). As such, the present invention encompasses soluble receptors that comprise portions of IL-17RC in combination with IL-17RA, IL-17RE or any other IL-17 family receptor. IL-17RC can also act as a heterodimeric receptor subunit for a IL-17-related cytokine. For instance, IL-17RC may form a heterodimer with IL-17RA or another IL-17-like receptor. IL-17RC is disclosed in commonly owned US Patent Application No. 10/458,647, and commonly owned WIPO publication WO 01/04304, both of which are incorporated herein in their entirety by reference. Analysis of a human cDNA clone encoding IL-17RC (SEQ ID NO:1) revealed an open reading frame encoding 692 amino acids (SEQ ID NO:2) comprising a putative signal sequence of approximately 20 amino acid residues (amino acird residues 1 to 20 of SEQ ID NO:2), an extracellular ligand-binding domain of approximately 431 amino acid residues (amino acid residues 21-452 of SEQ ID NO:2; SEQ ID NO:3), a transmembrane domain of approximately 20 amino acid residues (amino acid residues 453-473 of SEQ ID NO:2), and an intracellular domain of approximately 203 amino acid residues (amino acid residues 474 to 677 of SEQ ID NO:2). Furthermore, a ligand binding domain is represented by SEQ ID NO:22.

[56] Yet another illustrative nucleotide sequence that encodes a variant human IL-17RC, designated as "IL-17RCx4" is provided by SEQ ID NO:165, the encoded polypeptide is shown in SEQ ID NO:166. The predicted signal peptides is from residues 1-60 of SEQ ID NO:165 and 1-20 of SEQ ID NO:166; the extracellular domain from residues 61-1401 of SEQ ID NO:165 and 21-467 of SEQ ID NO:166; the transmembrane domain is from residues 1402-1464 of SEQ ID NO:165 and 468-488 of SEQ ID NO:166; and the intracellular domain is from residues 1465-2121 of SEQ ID NO:165 and 489-707 of SEQ ID NO:166.

[57] Yet another illustrative nucleotide sequence that encodes a variant human IL-17RC, designated as "IL-17RC-1" is provided by SEQ ID NO:4, the encoded polypeptide is shown in SEQ ID NO:5. IL-17RC-1 is disclosed in commonly owned US Patent Application No. 10/458,647, and commonly owned WIPO publication WO 01/04304, both of which are incorporated herein in their

entirety by reference. Sequence analysis revealed that IL-17RC-1 is a truncated form of receptor polypeptide. That is, IL-17RC-1 lacks amino acid residues 1-113 of SEQ ID NO:2. SEQ ID NO:10 presents an amino acid sequence of a IL-17RC-1 polypeptide that includes the N-terminal portion of IL-17RC.

[58] A comparison of the IL-17RC and IL-17RC-1 amino acid sequences also indicated that the two polypeptides represent alternatively spliced variants. The amino acid sequence of IL-17RC includes a 17 amino acid segment (amino acid residues 339 to 355 of SEQ ID NO:2), which IL-17RC-1 lacks, while IL-17RC lacks, following amino acid 479, a 13 amino acid segment found in IL-17RC-1 (amino acid residues 350 to 362 of SEQ ID NO:5). A polypeptide that contains both amino acid segments is provided by SEQ ID NO:11, whereas SEQ ID NO:12 presents the amino acid sequence of a polypeptide that lacks both 13 and 17 amino acid segments.

[59] Yet another illustrative nucleotide sequence that encodes a variant human IL-17RC, designated as "IL-17RC-6" is provided by SEQ ID NO:23, the encoded polypeptide is shown in SEQ ID NO:24. IL-17RC-6 contains a 25 amino acid residue deletion as compared to IL-17RC as embodied in SEQ ID NO:2. Specifically, IL-17RC-6 does not contain amino acid residue 94 to amino acid residue 118 of SEQ ID NO:2. Analysis of a human cDNA clone encoding IL-17RC-6 (SEQ ID NO:23) revealed an extracellular ligand-binding domain of approximately 427 amino acid residues (amino acid residues 1-427 of SEQ ID NO:24), a transmembrane domain of approximately 20 amino acid residues (amino acid residues 428-448 of SEQ ID NO:24), and an intracellular domain of approximately 218 amino acid residues (amino acid residues 449 to 667 of SEQ ID NO:24).

[60] An illustrative nucleotide sequence that encodes a variant murine IL-17RC is provided by SEQ ID NO:25; the encoded polypeptide is shown in SEQ ID NO:26. Murine IL-17RC functions as a receptor for both murine IL-17A (SEQ ID NOS:17 & 18) and murine IL-17F (SEQ ID NOS:19 & 20). Analysis of a murine cDNA clone encoding IL-17RC (SEQ ID NO:25) revealed an extracellular ligand-binding domain of approximately 449 amino acid residues SEQ ID NO:27). Furthermore, a ligand binding domain is represented by SEQ ID NO:28.

[61] Yet another illustrative nucleotide sequence that encodes a variant murine IL-17RC is provided by SEQ ID NO:29; the encoded polypeptide is shown in SEQ ID NO:30.

[62] The IL-17RC gene resides in chromosome 3p25 – 3p24. As discussed below, this region is associated with various disorders and diseases.

[63] Northern analyses indicate that there is strong expression of the IL-17RC gene in thyroid, adrenal gland, prostate, and liver tissues, and less expression in heart, small intestine, stomach, and trachea tissues. In contrast, there is little or no expression in brain, placenta, lung, skeletal muscle, kidney, pancreas, spleen, thymus, testis, ovary, colon, peripheral blood leukocytes, spinal cord, lymph node, and bone marrow. These observations show that IL-17RC sequences can be used differentiate between various tissues.

[64] As described below, the present invention provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90%, or greater than 95%, such as 96%, 97%, 98%, or greater than 99% or more identical to a reference amino acid sequence of 21-692 of SEQ ID NO:2, wherein the isolated polypeptide specifically binds with an antibody that specifically binds with a polypeptide comprising the amino acid sequence of SEQ ID NO:2. The present invention also provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90% identical to a reference amino acid sequence selected from the group consisting of: (a) amino acid residues 21 to 452 of SEQ ID NO:2, (b) amino acid residues 21 to 435 of SEQ ID NO:10, (c) amino acid residues 21 to 677 of SEQ ID NO:2, and (d) amino acid residues 1 to 692 of SEQ ID NO:2, wherein the isolated polypeptide specifically binds with an antibody that specifically binds with a polypeptide consisting of either the amino acid sequence of SEQ ID NO:2, or the amino acid sequence of SEQ ID NO:10. Illustrative polypeptides include a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

[65] The present invention also provides isolated polypeptides comprising an extracellular domain, wherein the extracellular domain comprises either amino acid residues 21 to 452 of the amino acid sequence of SEQ ID NO:2 or amino acid residues 21 to 435 of the amino acid sequence of SEQ ID NO:10. Such polypeptides may further comprise a transmembrane domain that resides in a carboxyl-terminal position relative to the extracellular domain, wherein the transmembrane domain comprises amino acid residues 453 to 473 of SEQ ID NO:2. These polypeptides may also comprise an intracellular domain that resides in a carboxyl-terminal position relative to the transmembrane domain, wherein the intracellular domain comprises either amino acid residues 474 to 677 of SEQ ID NO:2, or amino acid residues 457 to 673 of SEQ ID NO:10, and optionally, a signal secretory sequence that resides in an amino-terminal position relative to the extracellular domain, wherein the signal secretory sequence comprises amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO:2.

[66] The present invention also includes variant IL-17RC polypeptides, wherein the amino acid sequence of the variant polypeptide shares an identity with the amino acid sequence of SEQ ID NO:2 selected from the group consisting of at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, or greater than 95% identity, and wherein any difference between the amino acid sequence of the variant polypeptide and the amino acid sequence of SEQ ID NO:2 is due to one or more conservative amino acid substitutions.

[67] Moreover, the present invention also provides isolated polypeptides as disclosed above that bind IL-17F (e.g., human IL-17F polypeptide sequence as shown in SEQ ID NO:16). The human IL-17F polynucleotide sequence is shown in SEQ ID NO:15. The mouse IL-17F polynucleotide sequence is shown in SEQ ID NO:19, and corresponding polyepeptide is shown in

SEQ ID NO:20. The present invention also provides isolated polypeptides as disclosed above that bind IL-17A (e.g., human IL-17A polypeptide sequence as shown in SEQ ID NO:14). The human IL-17A polynucleotide sequence is shown in SEQ ID NO:13. The mouse IL-17A polynucleotide sequence is shown in SEQ ID NO:17, and corresponding polyepeptide is shown in SEQ ID NO:18.

[68] The present invention also provides isolated polypeptides and epitopes comprising at least 15 contiguous amino acid residues of an amino acid sequence of SEQ ID NO:2 or 3. Illustrative polypeptides include polypeptides that either comprise, or consist of SEQ ID NO:2 or 3, an antigenic epitope thereof, or a functional IL-17A or IL-17F binding fragment thereof. Moreover, the present invention also provides isolated polypeptides as disclosed above that bind to, block, inhibit, reduce, antagonize or neutralize the activity of IL-17F or IL-17A.

[69] The present invention also includes variant IL-17RC polypeptides, wherein the amino acid sequence of the variant polypeptide shares an identity with the amino acid residues of SEQ ID NO:2 selected from the group consisting of at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, or greater than 95% identity, such as 96%, 97%, 98%, or greater than 99% or more identity, and wherein any difference between the amino acid sequence of the variant polypeptide and the corresponding amino acid sequence of SEQ ID NO:2 is due to one or more conservative amino acid substitutions. Such conservative amino acid substitutions are described herein. Moreover, the present invention also provides isolated polypeptides as disclosed above that bind to, block, inhibit, reduce, antagonize or neutralize the activity of IL-17F or IL-17A.

[70] The present invention further provides provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier and at least one of such an expression vector or recombinant virus comprising such expression vectors. The present invention further includes pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and a polypeptide or antibody described herein.

[71] The present invention also provides fusion proteins, comprising a IL-17RC polypeptide and an immunoglobulin moiety. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F_c fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins.

[72] These and other aspects of the invention will become evident upon reference to the following detailed description. In addition, various references are identified below and are incorporated by reference in their entirety.

B) Definitions

[73] In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

[74] As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*, α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

[75] The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

[76] The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

[77] The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

[78] An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

[79] A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

[80] "Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

[81] "Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

[82] A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol.* 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al.*, *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye *et al.*, *J. Biol. Chem.* 269:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed.. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

[83] A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

[84] A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

[85] An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

[86] "Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.,* endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.,* exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

[87] A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

[88] A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[89] A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

[90] A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

[91] An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

[92] A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces IL-17RC from an expression vector. In contrast, IL-17RC can be produced by a cell that is a "natural source" of IL-17RC, and that lacks an expression vector.

[93] "Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

[94] A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a IL-17RC polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of IL-17RC using affinity chromatography.

[95] The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

[96] In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

[97] A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains, and other linkage to the cell membrane such as via glycophosphoinositol (gpi). Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis or translated from alternatively spliced mRNAs. Soluble receptors can be monomeric, homodimeric, heterodimeric, or multimeric, with multimeric receptors generally not comprising more than 9 subunits, preferably not comprising more than 6 subunits, and most preferably not comprising more than 3 subunits. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments

when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively. Soluble receptors of cytokine receptors generally comprise the extracellular cytokine binding domain free of a transmsmbrane domain and intracellular domain. For example, representative soluble receptors include soluble receptors for IL-17RA as shown in SEQ ID NOs: 167 (polynucleotide) and 21 (polypeptide). It is well within the level of one of skill in the art to delineate what sequences of a known cytokine receptor sequence comprise the extracellular cytokine binding domain free of a transmsmbrane domain and intracellular domain. Moreover, one of skill in the art using the genetic code can readily determine polynucleotides that encode such soluble receptor polyptides.

[98] The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

[99] An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.,* at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, such as 96%, 97%, or 98% or more pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

[100] The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

[101] The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

[102] The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may

encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

[103] As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, an dthe like, and synthetic analogs of these molecules.

[104] The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

[105] An "anti-idiotype antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotype antibody binds with the variable region of an anti-IL-17RC antibody, and thus, an anti-idiotype antibody mimics an epitope of IL-17RC.

[106] An "antibody fragment" is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-IL-17RC monoclonal antibody fragment binds with an epitope of IL-17RC.

[107] The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

[108] A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

[109] "Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain. Construction of humanized antibodies for therapeutic use in humans that are derived from murine antibodies, such as those that bind to or neutralize a human protein, is within the skill of one in the art.

[110] As used herein, a "therapeutic agent" is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for therapy. Examples of therapeutic agents

include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

[111] A "detectable label" is a molecule or atom which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

[112] The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al., EMBO J. 4*:1075 (1985); Nilsson *et al., Methods Enzymol. 198*:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene 67*:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952 (1985)), substance P, FLAG peptide (Hopp *et al., Biotechnology 6*:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification 2*:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.,* Pharmacia Biotech, Piscataway, NJ).

[113] A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

[114] As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

[115] An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

[116] As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a IL-17RC polypeptide component. Examples of an antibody fusion protein include a protein that comprises a IL-17RC extracellular domain, and either an Fc domain or an antigen-binding region.

[117] A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

[118] An "antigenic peptide" is a peptide which will bind a major histocompatibility complex molecule to form an MHC-peptide complex which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a

cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

[119] In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an "anti-sense gene." Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

[120] An "anti-sense oligonucleotide specific for IL-17RC" or a "IL-17RC anti-sense oligonucleotide" is an oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the *IL-17RC* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *IL-17RC* gene.

[121] A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a ribozyme is termed a "ribozyme gene."

[122] An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

[123] The term "variant IL-17RC gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:2. Such variants include naturally-occurring polymorphisms of IL-17RC genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:2. Additional variant forms of IL-17RC genes are nucleic acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant IL-17RC gene can be identified, for example, by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 OR SEQ ID NO:4, or its complement, under stringent conditions.

[124] Alternatively, variant IL-17RC genes can be identified by sequence comparison. Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have "100% nucleotide sequence identity" if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other

methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu *et al.* (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

[125] Regardless of the particular method used to identify a variant *IL*-17RC gene or variant IL-17RC polypeptide, a variant gene or polypeptide encoded by a variant gene may be functionally characterized the ability to bind specifically to an anti-IL-17RC antibody. A variant IL-17RC gene or variant IL-17RC polypeptide may also be functionally characterized the ability to bind to its ligand, for example, IL-17A and/or IL-17F, using a biological or biochemical assay described herein.

[126] The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

[127] The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

[128] "Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

[129] The present invention includes functional fragments of IL-17RC genes. Within the context of this invention, a "functional fragment" of a IL-17RC gene refers to a nucleic acid molecule that encodes a portion of a IL-17RC polypeptide which is a domain described herein or at least specifically binds with an anti-IL-17RC antibody.

[130] Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

C) Production of IL-17RA and IL-17RC Polynucleotides or Genes

[131] Nucleic acid molecules encoding a human IL-17RA or IL-17RC gene or polynucleotides encoding any of the soluble polypeptides of the present invention can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon SEQ ID NO:1,

SEQ ID NO:4. These techniques are standard and well-established, and may be accomplished using cloning kits available by commercial suppliers. See, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology, 3rd Edition*, John Wiley & Sons 1995; Wu *et al.*, *Methods in Gene Biotechnology*, CRC Press, Inc. 1997; Aviv and Leder, *Proc. Nat'l Acad. Sci. USA 69:*1408 (1972); Huynh *et al.*, "Constructing and Screening cDNA Libraries in λgt10 and λgt11," in *DNA Cloning: A Practical Approach Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

[132] Nucleic acid molecules that encode a human IL-17RA or IL-17RC gene can also be obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the IL-17RA or IL-17RC gene or cDNA. General methods for screening libraries with PCR are provided by, for example, Yu *et al.*, "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), Humana Press, Inc., 1993. Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), Humana Press, Inc. 1993. As an alternative, an IL-17RA or IL-17RC gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995)). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol. 21:*1131 (1993), Bambot *et al.*, *PCR Methods and Applications 2:*266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al., PCR Methods Appl. 4:*299 (1995)). For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al., Annu. Rev. Biochem. 53:*323 (1984), and Climie *et al., Proc. Nat'l Acad. Sci. USA 87:*633 (1990).

D) Production of IL-17RA or IL-17RC Gene Variants

[133] The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, that encode the IL-17RA or IL-17RC polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. Moreover, the present invention also provides isolated soluble monomeric, homodimeric, heterodimeric and multimeric receptor polypeptides that comprise at least a portion of IL-17RC that is substantially homologous to the receptor polypeptide of SEQ ID NO:2. Thus, the present invention contemplates

IL-17RA or IL-17RC polypeptide-encoding nucleic acid molecules comprising degenerate nucleotides of SEQ ID NO:1 or SEQ ID NO:4, and their RNA equivalents.

[134] Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:7 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the IL-17RC polypeptide of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:7 also provides all RNA sequences encoding SEQ ID NO:2,. by substituting U for T. Thus, the present invention contemplates IL-17RC polypeptide-encoding nucleic acid molecules comprising nucleotide 154 to nucleotide 2229 of SEQ ID NO:1, and their RNA equivalents. Similarly, the IL-17RC-1 degenerate sequence of SEQ ID NO:6 also provides all RNA sequences encoding SEQ ID NO:5, by substituting U for T.

[135] Table 4 sets forth the one-letter codes to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

Table 4

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

[136] The degenerate codons, encompassing all possible codons for a given amino acid, are set forth in Table 5.

Table 5

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

[137] One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For

example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NO:6. Variant sequences can be readily tested for functionality as described herein.

[138] Different species can exhibit "preferential codon usage." In general, see, Grantham *et al., Nucl. Acids Res. 8*:1893 (1980), Haas *et al. Curr. Biol. 6*:315 (1996), Wain-Hobson *et al., Gene 13*:355 (1981), Grosjean and Fiers, *Gene 18*:199 (1982), Holm, *Nuc. Acids Res. 14*:3075 (1986), Ikemura, *J. Mol. Biol. 158*:573 (1982), Sharp and Matassi, *Curr. Opin. Genet. Dev. 4*:851 (1994), Kane, *Curr. Opin. Biotechnol. 6*:494 (1995), and Makrides, *Microbiol. Rev. 60*:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 5). For example, the amino acid threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed herein serve as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

[139] An IL-17RA or IL-17RC-encoding cDNA can be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative human IL-17RA or IL-17RC sequences disclosed herein. In addition, a cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to IL-17RA or IL-17RC polypeptide.

[140] Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human IL-17RC, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the

nucleotide sequences disclosed herein, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of the amino acid sequences disclosed herein. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the IL-17RC polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

[141] Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides encoding a soluble receptor that comprises a portion of an IL-17RC receptor subunit that is substantially homologous to either SEQ ID NO:1 or SEQ ID NO:4, or that encodes all of or a fragment of SEQ ID NO:2 or SEQ ID NO:5, or allelic variants thereof and retain the ligand-binding properties of the wild-type IL-17RC receptor. Such polypeptides may also include additional polypeptide segments as generally disclosed herein.

[142] Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. For example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1 OR SEQ ID NO:4, or to nucleic acid molecules comprising a nucleotide sequence complementary to SEQ ID NO:1 OR SEQ ID NO:4, or fragments thereof.

[143] In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. See, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.,* (eds.), *Current Protocols in Molecular Biology (*John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user-defined criteria. It is well within the abilities of one skilled in the art to adapthybridization and wash conditions for use with a particular polynucleotide hybrid.

[144] The present invention also provides isolated IL-17RA or IL-17RC polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NO:2 (IL-17RC) and

SEQ ID NO:21 (IL-17RA), or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides having at least 70%, at least 80%, at least 90%, at least 95%, such as 96%, 97%, 98%, or greater than 95% sequence identity to the sequences shown in SEQ ID NO:2, or their orthologs. For example, variant and orthologous IL-17RA or IL-17RC receptors can be used to generate an immune response and raise cross-reactive antibodies to human IL-17RA or IL-17RC. Such antibodies can be humanized, and modified as described herein, and used therauputically to treat psoriasis, psoriatic arthritis, IBD, IBS, colitis, endotoxemia as well as in other therapeutic applications described herein.

[145] The present invention also contemplates IL-17RA or IL-17RC variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with the amino acid sequence of SEQ ID NO:2 (IL-17RC) or SEQ ID NO:21 (IL-17RA), and a hybridization assay. Such variants include nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4 for IL-17RC (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95%, or greater than 95% such as 96%, 97%, 98%, or 99%, sequence identity to the amino acid sequence of SEQ ID NO:2. Alternatively, IL-17RC variants can be characterized as nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 OR SEQ ID NO:4 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95%, such as 96%, 97%, 98%, or 99% or greater, sequence identity to the amino acid sequence of SEQ ID NO:2.

[146] Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al., Bull. Math. Bio. 48*:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 6 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences])(100).

Table 6

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

[147] Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative IL-17RC variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2 or SEQ ID NO:3) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are

several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

[148] FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

[149] The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with an amino acid sequence disclosed herein. For example, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NO:2 or 21, in which an alkyl amino acid is substituted for an alkyl amino acid in a IL-17RA or IL-17RC amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a IL-17RA or IL-17RC amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a IL-17RA or IL-17RC amino acid sequence, a hydroxy-containing amino acid is substituted for a hydroxy-containing amino acid in a IL-17RA or IL-17RC amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a IL-17RA or IL-17RC amino acid sequence, a basic amino acid is substituted for a basic amino acid in a IL-17RA or IL-17RC amino acid sequence, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a IL-17RA or IL-17RC amino acid sequence. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine. The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the

language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (*e.g.*, 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (*e.g.*, 2 or 3).Particular variants of IL-17RC are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% such as 96%, 97%, 98%, or 99% or greater sequence identity to the corresponding amino acid sequence (*e.g.*, SEQ ID NO:2 or 21), wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

[150] Conservative amino acid changes in a IL-17RA or IL-17RC gene can be introduced, for example, by substituting nucleotides for the nucleotides recited in SEQ ID NO:1 or SEQ ID NO:4. Such "conservative amino acid" variants can be obtained by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel (1995); and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). A variant IL-17RC polypeptide can be identified by the ability to specifically bind anti-IL-17RC antibodies.

[151] The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al., J. Am. Chem. Soc. 113*:2722 (1991), Ellman *et al., Methods Enzymol. 202*:301 (1991), Chung *et al., Science 259*:806 (1993), and Chung *et al., Proc. Nat'l Acad. Sci. USA 90*:10145 (1993).

[152] In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al., J. Biol. Chem. 271*:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally

occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide *et al., Biochem. 33*:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci. 2*:395 (1993)).

[153] A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for IL-17RA or IL-17RC amino acid residues.

[154] Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081 (1989), Bass *et al., Proc. Nat'l Acad. Sci. USA 88*:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

[155] Although sequence analysis can be used to further define the IL-17RA or IL-17RC ligand binding region, amino acids that play a role in IL-17RA or IL-17RC binding activity (such as binding of IL-17RC to either Il-17A or IL-17F, and IL-17RA to IL-17A) can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992). Specifically, three domains were identified:

1) Domain 1 (SEQ ID NOs: 159 and 160) comprises exons 8-10 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 193-276 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 208-291 of (SEQ ID NO:166).

2) Domain 2 (SEQ ID NOs: 161 and 162) comprises exons 11-13 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 277-370 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 292-385 of (SEQ ID NO:166).

3) Domain 3 (SEQ ID NOs: 163 and 164) comprises exons 8-10 of IL-17RC. This corresponds to IL-17RCx1's amino acid residues 371-447 of (SEQ ID NO:2) and IL-17RCx4's amino acid residues 386-462 of (SEQ ID NO:166).

[156] Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science 241*:53

(1988)) or Bowie and Sauer (*Proc. Nat'l Acad. Sci. USA 86:*2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.,* Lowman *et al., Biochem. 30:*10832 (1991), Ladner *et al.,* U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire *et al., Gene 46:*145 (1986), and Ner *et al., DNA 7:*127, (1988)). Moreover, IL-17RC or IL-17RA labeled with biotin or FITC can be used for expression cloning of IL-17RC ligands.

[157] Variants of the disclosed IL-17RC or IL-17RA nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, *Nature 370:*389 (1994), Stemmer, *Proc. Nat'l Acad. Sci. USA 91:*10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

[158] Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti- IL-17RC or IL-17RA antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

[159] The present invention also includes "functional fragments" of IL-17RC or IL-17RA polypeptides and nucleic acid molecules encoding such functional fragments. These functional fragments may either bind ligand or ligands (i.e. both IL-17A and IL-17F) singly or together. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a IL-17RC or IL-17RA polypeptide. As an illustration, DNA molecules having the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4 can be digested with *Bal*31 nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to bind anti-IL-17RC antibodies. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired

fragment. Alternatively, particular fragments of a IL-17RC or IL-17RA gene can be synthesized using the polymerase chain reaction.

[160] This general approach is exemplified by studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther. 66*:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter *et al.*, *Molec. Gen. Genet. 240*:113 (1993), Content *et al.*, "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987), Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation, Vol. 1*, Boynton *et al.*, (eds.) pages 169-199 (Academic Press 1985), Coumailleau *et al.*, *J. Biol. Chem. 270*:29270 (1995); Fukunaga *et al.*, *J. Biol. Chem. 270*:25291 (1995); Yamaguchi *et al.*, *Biochem. Pharmacol. 50*:1295 (1995), and Meisel *et al.*, *Plant Molec. Biol. 30*:1 (1996).

[161] The present invention also contemplates functional fragments of a IL-17RC or IL-17RA gene that have amino acid changes, compared with an amino acid sequence disclosed herein. A variant IL-17RC or IL-17RA gene can be identified on the basis of structure by determining the level of identity with disclosed nucleotide and amino acid sequences, as discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant IL-17RC or IL-17RA gene can hybridize to a nucleic acid molecule comprising a nucleotide sequence, such as SEQ ID NO:1 or SEQ ID NO:4.

[162] The present invention also includes using functional fragments of IL-17RC or IL-17RA polypeptides, antigenic epitopes, epitope-bearing portions or ligand-binding portions of IL-17RC and/or IL-17RA polypeptides, and nucleic acid molecules that encode such functional fragments, antigenic epitopes, epitope-bearing portions or ligand-binding portions of IL-17RC and/or IL-17RA polypeptides. Such fragments are used to generate polypeptides for use in generating soluble receptors or binding molecules that bind, block, inhibit, reduce, antagonize or neutralize activity of IL-17A or IL-17F or both IL-17A and IL-17F. A "functional" IL-17RC or IL-17RC/IL-17RA polypeptide or fragment thereof as defined herein is characterized by its ability to block, inhibit, reduce, antagonize or neutralize IL-17A and/or IL-17F inflammatory, proliferative or differentiating activity, by its ability to induce or inhibit specialized cell functions, or by its ability to bind specifically to IL-17A and/or IL-17F. As previously described herein, both IL-17RA and IL-17RC is characterized by a unique cytokine receptor structure and domains as described herein. Thus, the present invention further contemplates using fusion proteins encompassing: (a) polypeptide molecules comprising one or more of the domains described above; and (b) functional fragments comprising one or more of these domains. The other polypeptide portion of the fusion protein may be contributed by another cytokine receptor, such as an IL-17-like receptor, IL-17RA, IL-17RE, IL-

17RD, or by a non-native and/or an unrelated secretory signal peptide that facilitates secretion of the fusion protein.

[163] The present invention also provides polypeptide fragments or peptides comprising an ligand-binding portion of a IL-17RC or IL-17RA polypeptide described herein. Such fragments or peptides may comprise a portion of either IL-17RC or IL-17RA that binds to its respective ligand (IL-17A and/or IL-17F).

[164] For any IL-17RC or IL-17RA polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above. Moreover, those of skill in the art can use standard software to devise IL-17RC or IL-17RA variants based upon the nucleotide and amino acid sequences described herein.

E) Production of IL-17RC, IL-17RA and IL-17RC/IL-17RA Polypeptides

[165] The polypeptides of the present invention, including full-length polypeptides; soluble monomeric, homodimeric, heterodimeric and multimeric receptors; full-length receptors; receptor fragments (e.g. ligand-binding fragments and antigenic epitopes), functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express an IL-17RC, IL-17RA and IL-17RC/IL-17RA gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector.

[166] Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, an IL-17RC expression vector may comprise an IL-17RC, IL-17RA and IL-17RC/IL-17RA gene and a secretory sequence derived from any secreted gene.

[167] IL-17RC, IL-17RA and IL-17RC/IL-17RA proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61;

CHO DG44 (Chasin *et al., Som. Cell. Molec. Genet. 12*:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

[168] For a mammalian host, the transcriptional and translational regulatory signals may be derived from mammalian viral sources, for example, adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, for example, actin, collagen, myosin, and metallothionein genes.

[169] Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al., J. Molec. Appl. Genet. 1*:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell 31*:355 (1982)), the *SV40* early promoter (Benoist *et al., Nature 290*:304 (1981)), the *Rous* sarcoma virus promoter (Gorman *et al., Proc. Nat'l Acad. Sci. USA 79*:6777 (1982)), the cytomegalovirus promoter (Foecking *et al., Gene 45*:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)).

[170] Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al., Mol. Cell. Biol. 10*:4529 (1990), and Kaufman *et al., Nucl. Acids Res. 19*:4485 (1991)).

[171] In certain embodiments, a DNA sequence encoding an IL-17RC, IL-17RA and IL-17RC/IL-17RA soluble receptor polypeptide, or a fragment of IL-17RC, IL-17RA or IL-17RC/IL-17RA polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers. Multiple components of a soluble receptor complex can be co-transfected on individual expression vectors or be contained in a single expression vector. Such techniques of expressing multiple components of protein complexes are well known in the art.

[172] An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel (1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

[173] For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase (DHFR), which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

[174] The polypeptides of the invention can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, retroviruses, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994), and Douglas and Curiel, *Science & Medicine 4*:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

[175] By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al., Cytotechnol. 15*:145 (1994)).

[176] The polypeptides of the invention can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned genes into insect cells. Suitable expression vectors are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein* (*hsp*) 70 promoter, *Autographa californica nuclear polyhedrosis virus immediate-early* gene promoter (*ie-1*) and the *delayed early 39K* promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al., J. Virol. 67*:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding a polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed the polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al., Proc. Nat'l Acad. Sci. 82*:7952 (1985)). Using a technique known in the art, a transfer vector containing a gene encoding a polypeptide of the present invention is transformed into *E. coli*, and screened for bacmids which contain an interrupted *lacZ* gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

[177] The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native IL-17RC secretory signal sequence.

[178] The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-*Sf*-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as *Sf*9 (ATCC CRL 1711), *Sf*21AE, and *Sf*21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH FIVEO cell line (Invitrogen) derived from *Trichoplusia*

ni (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

[179] Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.*, "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression Protocols*, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

[180] Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GAL1* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOX1* (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311, Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

[181] Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia*

pastoris, Pichia methanolica, Pichia guillermondii and *Candida maltosa* are known in the art. See, for example, Gleeson *et al., J. Gen. Microbiol. 132*:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.,* U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.,* U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

[182] For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al., Yeast 14*:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

[183] Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al., Science 227*:1229 (1985), Klein *et al., Biotechnology 10*:268 (1992), and Miki *et al.,* "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).

[184] Alternatively, genes encoding the polypeptides of the present invention can be expressed in prokaryotic host cells. Suitable promoters that can be used to express IL-17RC

polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the P_R and P_L promoters of bacteriophage lambda, the *trp*, *recA*, heat shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli,* promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, *J. Ind. Microbiol. 1*:277 (1987), Watson *et al., Molecular Biology of the Gene, 4th Ed.* (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

[185] Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilus*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilus* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

[186] When expressing a polypeptide of the present invention in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

[187] Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.,* "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.,* "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley & Sons, Inc. 1996)).

[188] Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

[189] General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, Grisshammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995).

[190] As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc. 85*:2149 (1963), Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot. 3*:3 (1986), Atherton *et al., Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al., Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al., Science 266*:776 (1994), Hackeng *et al., Proc. Nat'l Acad. Sci. USA 94*:7845 (1997), Dawson, *Methods Enzymol. 287*: 34 (1997), Muir *et al, Proc. Nat'l Acad. Sci. USA 95*:6705 (1998), and Severinov and Muir, *J. Biol. Chem. 273*:16205 (1998)).

[191] Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2, 5 or 21. As an illustration, polypeptides can comprise at least six, at least nine, or at least 15 contiguous amino acid residues of of SEQ ID NO:2, 5 and/or 21. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

[192] Moreover, the polypeptides and fragments thereof of the present invention can be expressed as monomers, homodimers, heterodimers, or multimers within higher eukaryotic cells. Such cells can be used to produce IL-17RC monomeric, homodimeric, heterodimeric and multimeric receptor polypeptides that comprise at least a portion of an IL-17RC polypeptide ("IL-17RC-comprising receptors" or "IL-17RC-comprising receptor polypeptides"), a portion of IL-17RC and IL-17RA together (as either a monomer, homodimer or heterodimer) or can be used as assay cells in screening systems. Within one aspect of the present invention, a polypeptide of the present invention

comprising at least the ligand-binding portion of either the IL-17RC or IL-17RA extracellular domain is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, IL-17F, as well as IL-17A, or even agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (*e.g.*, a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems. Each component of the monomeric, homodimeric, heterodimeric and multimeric receptor complex can be expressed in the same cell. Moreover, the components of the monomeric, homodimeric, heterodimeric and multimeric receptor complex can also be fused to a transmembrane domain or other membrane fusion moiety to allow complex assembly and screening of transfectants as described above.

[193] To assay polyepeptides of the present invention, mammalian cells suitable for use in expressing IL-17RC and IL-17RC/IL-17RA receptors or other receptors known to bind IL-17A or IL-17F (e.g., cells expressing IL-17R) and transducing a receptor-mediated signal include cells that express other receptor subunits that may form a functional complex with IL-17RC. It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation. Preferred cell lines of this type are the human TF-1 cell line (ATCC number CRL-2003) and the AML-193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines and BaF3 (Palacios and Steinmetz, *Cell 41*: 727-734, (1985)) which is an IL-3 dependent murine pre-B cell line. Other cell lines include BHK, COS-1 and CHO cells. Suitable host cells can be engineered to produce the necessary receptor subunits or other cellular component needed for the desired cellular response. This approach is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. Species orthologs of the human receptor cDNA can be cloned and used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF or IL-3, can thus be engineered to become dependent upon another cytokine that acts through the IL-17RC or IL-17RA receptor, such as IL-17F or IL-17A.

[194] Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, *J. Immunol. Meth.* 65: 55-63, (1983)). An alternative assay format uses cells that are further engineered to express a reporter

gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE. See, e.g., Shaw *et al.*, *Cell 56*:563-572, (1989). A preferred such reporter gene is a luciferase gene (de Wet *et al.*, *Mol. Cell. Biol. 7*:725, (1987)). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner *et al., J. Biol. Chem. 269*:29094-29101, (1994); Schenborn and Goiffin, *Promega_Notes 41*:11, 1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

[195] An additional screening approach provided by the present invention includes the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of IL-17RC, is joined to the ligand-binding domain of a second receptor. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of IL-17RC (SEQ ID NO:3) and IL-17RA (**SEQ ID NO:X**) with an intracellular domain of a second receptor, preferably a hematopoietic cytokine receptor, and a transmembrane domain. Such hybrid monomers, homodimers, heterodimers and multimers of the present invention receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the identification of a responsive cell type for the development of an assay for detecting IL-17F or IL-17A. Moreover, such cells can be used in the presence of IL-17F or IL-17A to assay the soluble receptor antagonists of the present invention in a competition-type assay. In such assay, a decrease in the proliferation or signal transduction activity of IL-17F or IL-17A in the presence of a soluble receptor of the present invention demonstrates antagonistic activity. Moreover IL-17RC-soluble receptor binding assays, an cell-based assays, can also be used to assess whether a soluble receptor binds, blocks, inhibits, reduces, antagonizes or neutralizes IL-17F or IL-17A activity.

F) Production of IL-17RC, IL-17RA and IL-17RC/IL-17RA Fusion Proteins and Conjugates

[196] One general class of IL-17RC, IL-17RA and IL-17RC/IL-17RA analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of IL-17RC, IL-17RA and IL-17RC/IL-17RA analogs is provided by anti-idiotype antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies

comprising anti-idiotype variable domains can be used as analogs (see, for example, Monfardini *et al., Proc. Assoc. Am. Physicians 108*:420 (1996)). Since the variable domains of anti-idiotype IL-17RC antibodies mimic IL-17RC, these domains can provide IL-17RC binding activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of skill in the art (see, for example, Joron *et al., Ann. N Y Acad. Sci. 672*:216 (1992), Friboulet *et al., Appl. Biochem. Biotechnol. 47*:229 (1994), and Avalle *et al., Ann. N Y Acad. Sci. 864*:118 (1998)).

[197] Another approach to identifying IL-17RC, IL-17RA and IL-17RC/IL-17RA analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press 1996), Verdine, U.S. Patent No. 5,783,384, Kay, *et. al.,* U.S. Patent No. 5,747,334, and Kauffman *et al.,* U.S. Patent No. 5,723,323.

[198] IL-17RC, IL-17RA and IL-17RC/IL-17RA polypeptides have both *in vivo* and *in vitro* uses. As an illustration, a soluble form of IL-17RC can be added to cell culture medium to inhibit the effects of the IL-17RC ligand (*i.e.* IL-17F, IL-17A or both) produced by the cultured cells.

[199] Fusion proteins of IL-17RC, IL-17RA and IL-17RC/IL-17RA can be used to express and isolate the corresponding polypeptide. As described below, particular IL-17RC, IL-17RA and IL-17RC/IL-17RA fusion proteins also have uses in diagnosis and therapy. One type of fusion protein comprises a peptide that guides a IL-17RC polypeptide from a recombinant host cell. To direct a IL-17RC polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pre sequence) is provided in the IL-17RC expression vector. While the secretory signal sequence may be derived from IL-17RC, a suitable signal sequence may also be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to a IL-17RC-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, *e.g.,* Welch *et al.,* U.S. Patent No. 5,037,743; Holland *et al.,* U.S. Patent No. 5,143,830).

[200] Although the secretory signal sequence of IL-17RC, IL-17RA and IL-17RC/IL-17RA as produced by mammalian cells (*e.g.,* tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of the corresponding polypeptide in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating phermone α-factor (encoded by the *MFα1* gene), invertase (encoded by the *SUC2* gene), or acid phosphatase (encoded by the *PHO5* gene). See, for example, Romanos *et al.*, "Expression of Cloned

Genes in Yeast," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

[201] The soluble receptor polypeptides of the present invention can be prepared by expressing a truncated DNA encoding the extracellular domain, for example, a polypeptide which contains all or a portion SEQ ID NO:3, or the corresponding region of a non-human receptor. It is preferred that the extracellular domain polypeptides be prepared in a form substantially free of transmembrane and intracellular polypeptide segments. To direct the export of the receptor domain from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide, such as a t-PA secretory peptide. To facilitate purification of the secreted receptor domain, a C-terminal extension, such as a poly-histidine tag, substance P, Flag™ peptide (Hopp *et al., Biotechnology 6*:1204-1210, (1988); available from Eastman Kodak Co., New Haven, CT) or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

[202] In an alternative approach, a receptor extracellular domain or portion thereof of IL-17RC, IL-17RA or IL-17RC/IL-17RA together can be expressed as a fusion with immunoglobulin heavy chain constant regions, typically an F_c fragment, which contains two constant region domains and a hinge region but lacks the variable region (See, Sledziewski, AZ et al., US Patent No. 6,018,026 and 5,750,375). The soluble polypeptides of the present invention include such fusions. One such fusion is shown in SEQ ID NO:64. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two receptor polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify the cognate ligand from solution, as an *in vitro* assay tool, to block, inhibit or reduce signals *in vitro* by specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, an IL-17RC, IL-17RA and IL-17RC/IL-17RA-Ig chimera is added to a sample containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A, which is immobilized on a solid support (e.g., insoluble resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution carried out as above. The chimeras may be used *in vivo* to regulate inflammatory responses including acute phase responses such as serum amyloid A (SAA), C-reactive protein (CRP), and the like. Chimeras with high binding affinity are administered parenterally (e.g., by intramuscular, subcutaneous or intravenous injection). Circulating molecules bind ligand and are cleared from circulation by normal physiological processes. For use in assays, the chimeras are bound to a support via the F_c region and used in an ELISA format.

[203] To assist in isolating polypeptides of the present invention, an assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/ anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. Alternatively, ligand/receptor binding can be analyzed using SELDI(TM) technology (Ciphergen, Inc., Palo Alto, CA).

[204] Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

[205] The present invention further provides a variety of other polypeptide fusions and related multimeric proteins comprising one or more polypeptide fusions. For example, a soluble IL-17RC, IL-17RA or IL-17RC/IL-17RA receptor polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains, e.g., IgGγ1, and the human κ light chain. Immunoglobulin-soluble fusions of the present invention can be expressed in genetically engineered cells to produce a variety of multimeric IL-17RC, IL-17RA or IL-17RC/IL-17RA receptor analogs. Auxiliary domains can be fused to soluble polypeptides of the present invention to target them to specific cells, tissues, or macromolecules (e.g., collagen, or cells expressing IL-17F or IL-17A). The polypeptides of the present invention can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connective Tissue Research 34:1-9, 1996.

[206] In bacterial cells, it is often desirable to express a heterologous protein as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, IL-17RC (or any polypeptide of the present invention) can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferease fusion proteins are

typically soluble, and easily purifiable from *E. coli* lysates on immobilized glutathione columns. In similar approaches, a IL-17RC fusion protein comprising a maltose binding protein polypeptide can be isolated with an amylose resin column, while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams *et al.*, "Expression of Foreign Proteins in *E. coli* Using Plasmid Vectors and Purification of Specific Polyclonal Antibodies," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press 1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

[207] Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyHistidine tags (which have an affinity for nickel-chelating resin), *c-myc* tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds with anti-FLAG antibodies). See, for example, Luo *et al., Arch. Biochem. Biophys. 329*:215 (1996), Morganti *et al., Biotechnol. Appl. Biochem. 23*:67 (1996), and Zheng *et al., Gene 186*:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

Another form of fusion protein comprises a polypeptide of the present invention and an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains two or three constant region domains and a hinge region but lacks the variable region. As an illustration, Chang *et al.*, U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment. The C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. An exemplary peptide linker has the amino acid sequence: GGSGG SGGGG SGGGG S (SEQ ID NO:9). In this fusion protein, an illustrative Fc moiety is a human γ4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a IL-17RC or an IL-17RC/IL-17RA fusion protein that comprises a IL-17RC or an IL-17RC and IL-17RA moiety and a human Fc fragment, wherein the C-terminus of the IL-17RC moiety is attached to the N-terminus of the Fc fragment via a peptide linker, such as a peptide comprising at least a portion of the amino acid sequence of SEQ ID NO:2, 5 or 21. Both the IL-17RC and the IL-17RA moiety can be the extracellualr domain or any fragment thereof. For example, a fusion protein can comprise the amino acid of SEQ ID NO:3 and an Fc fragment (*e.g.*, a human Fc fragment) (SEQ ID NO:64). Another example of such a fusion protein is Variant 1454 (SEQ ID NOs: 157 and 158) which includes exons

1-6 of human IL-17RA and 8-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant 1454 also has the native signal peptide from human IL-17RA. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

[208] In another variation, a fusion protein of the present invention comprises an IgG sequence, an IL-17RC, IL-17RA or IL-17RC/IL-17RA moiety covalently joined to the aminoterminal end of the IgG sequence, and a signal peptide that is covalently joined to the aminoterminal of the IL-17RC or IL-17RA moiety, wherein the IgG sequence consists of the following elements in the following order: a hinge region, a CH_2 domain, and a CH_3 domain. Accordingly, the IgG sequence lacks a CH_1 domain. These moieties should display a biological activity, as described herein, such as the ability to bind with IL-17A and/or IL-17F. This general approach to producing fusion proteins that comprise both antibody and nonantibody portions has been described by LaRochelle *et al.*, EP 742830 (WO 95/21258).

[209] Fusion proteins comprising a IL-17RC or IL-17RC/IL-17RA moiety and an Fc moiety can be used, for example, as an *in vitro* assay tool. For example, the presence of IL-F in a biological sample can be detected using a IL-17RC-immunoglobulin fusion protein, in which the IL-17RC moiety is used to bind the ligand, and a macromolecule, such as Protein A or anti-Fc antibody, is used to bind the fusion protein to a solid support. Such systems can be used to identify agonists and antagonists that interfere with the binding of a IL-17 family ligands, e.g., IL-17F or both IL-17A and IL-17F, to their receptor.

[210] The present invention further provides a variety of other polypeptide fusions. For example, part or all of a domain(s) conferring a desired biological function (eg. Binding IL-17A) can be added to a portion of IL-17RC with the functionally equivalent domain(s) from another member of the cytokine receptor family (i.e. IL-17RA) to create a different molecule (i.e. IL-17RC/IL-17RA). Polypeptide fusions can be expressed in recombinant host cells to produce a variety of these fusion analogs. An IL-17RC, IL-17RA or IL-17RC/IL-17RA polypeptide can be fused to two or more moieties or domains, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, for example, Tuan *et al., Connective Tissue Research 34*:1 (1996).

[211] Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. General methods for enzymatic and chemical cleavage of fusion proteins are described, for example, by Ausubel (1995) at pages 16-19 to 16-25.

[212] IL-17RC and/or IL-17RA binding domains can be further characterized by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance,

crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids of ligand agonists. See, for example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

[213] The present invention also contemplates chemically modified IL-17RC or IL-17RC/IL-17RA compositions, in which the polypeptide is linked with a polymer. Illustrative IL-17RC or IL-17RC/IL-17RA polypeptides are soluble polypeptides that lack a functional transmembrane domain, such as a polypeptide consisting of amino acid residues SEQ ID NO:3 or **X**. Typically, the polymer is water soluble so that the conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation. In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C1-C10) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce IL-17RC or IL-17RC/IL-17RA conjugates.

[214] The conjugates of the present invention used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C1-C10)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.,* glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A IL-17RC conjugate can also comprise a mixture of such water-soluble polymers.

[215] One example of a IL-17RC conjugate comprises a IL-17RC moiety (or an IL-17RC/IL-17RA moiety) and a polyalkyl oxide moiety attached to the *N*-terminus of the IL-17RC moiety. PEG is one suitable polyalkyl oxide. As an illustration, IL-17RC (or IL-17RC/IL-17RA) can be modified with PEG, a process known as "PEGylation." PEGylation of IL-17RC can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al., Critical Reviews in Therapeutic Drug Carrier Systems 9*:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet. 27*:290 (1994), and Francis *et al., Int J Hematol 68*:1 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, IL-17RC conjugates are formed by condensing activated PEG, in which a terminal hydroxy or amino group of PEG has been replaced by an activated linker (see, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

[216] PEGylation by acylation typically requires reacting an active ester derivative of PEG with a IL-17RC or IL-17RC/IL-17RA polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between IL-17RC or IL-17RC/IL-17RA and a water soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated IL-17RC or IL-17RC/IL-17RA by acylation will typically comprise the steps of (a) reacting a IL-17RC or IL-17RC/IL-17RA polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to IL-17RC or IL-17RC/IL-17RA, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:IL-17RC (or PEG:IL-17RC/IL-17RA), the greater the percentage of polyPEGylated IL-17RC (or IL-17RC/IL-17RA) product.

[217] The product of PEGylation by acylation is typically a polyPEGylated product, wherein the lysine ε-amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting IL-17RC or IL-17RC/IL-17RA will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated IL-17RC or IL-17RC/IL-17RA polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

[218] PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with IL-17RC or IL-17RC/IL-17RA in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a $-CH_2-NH$ group.

[219] Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the ε-amino groups of the lysine residues and the α-amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of IL-17RC or IL-17RC/IL-17RA monopolymer conjugates.

[220] Reductive alkylation to produce a substantially homogenous population of monopolymer IL-17RC or IL-17RC/IL-17RA conjugate molecule can comprise the steps of: (a) reacting a IL-17RC or IL-17RC/IL-17RA polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the α-amino group at the amino

terminus of the IL-17RC or IL-17RC/IL-17RA, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the Schiff base formed in the initial process of reductive alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

[221] For a substantially homogenous population of monopolymer IL-17RC or IL-17RC/IL-17RA conjugates, the reductive alkylation reaction conditions are those that permit the selective attachment of the water-soluble polymer moiety to the *N*-terminus of IL-17RC or IL-17RC/IL-17RA. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the α-amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal α-group, the more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:IL-17RC (or polymer:IL-17RC/IL-17RA) need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6. This method can be employed for making IL-17RC or IL-17RC/IL-17RA-comprising homodimeric, heterodimeric or multimeric soluble receptor conjugates.

[222] Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to IL-17RC or IL-17RC/IL-17RA will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to IL-17RC or IL-17RC/IL-17RA will be 1:1 to 20:1 for polyPEGylation, and 1:1 to 5:1 for monoPEGylation.

[223] General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al., Clin. Pharmacol. Ther. 59*:636 (1996), Monkarsh *et al., Anal. Biochem. 247*:434 (1997)). This method can be employed for making IL-17RC-comprising homodimeric, heterodimeric or multimeric soluble receptor conjugates.

[224] The present invention contemplates compositions comprising a peptide or polypeptide, such as a soluble receptor or antibody described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

G) Isolation of IL-17RC or IL-17RC/IL-17RA Polypeptides

[225] The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95%, such as 96%, 97%, 98%, or greater than 99% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

[226] Fractionation and/or conventional purification methods can be used to obtain preparations of IL-17RC or IL-17RC/IL-17RA purified from natural sources (*e.g.,* human tissue sources), synthetic IL-17RC or IL-17RC/IL-17RA polypeptides, and recombinant IL-17RC or IL-17RC/IL-17RA polypeptides and fusion IL-17RC or IL-17RC/IL-17RA polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

[227] Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988), and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

[228] Additional variations in IL-17RC or IL-17RC/IL-17RA isolation and purification can be devised by those of skill in the art.

[229] The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem. 3*:1 (1985)). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol. 182*:529 (1990)). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (*e.g.,* maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification. Moreover, the ligand-binding properties of the soluble IL-17RC or IL-17RC/IL-17RA polypeptides of the present invention can be exploited for purification, for example, of IL-17RC-comprising soluble receptors; for example, by using affinity chromatography wherein IL-17F ligand is bound to a column and the IL-17RC-comprising receptor is bound and subsequently eluted using standard chromatography methods.

[230] IL-17RC, IL-17RA or IL-17RC/IL-17RA polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. These polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue.

H) Production of Antibodies to IL-17RC or IL-17RC/IL-17RA Proteins

[231] Antibodies to IL-17RC or IL-17RC/IL-17RA can be obtained, for example, using the product of a IL-17RC or IL-17RC/IL-17RA expression vector or IL-17RC or IL-17RC/IL-17RA isolated from a natural source as an antigen. Particularly useful anti-IL-17RC or IL-17RC/IL-17RA antibodies "bind specifically" with IL-17RC or IL-17RC/IL-17RA. Antibodies are considered to be specifically binding if the antibodies exhibit at least one of the following two properties: (1) antibodies bind to IL-17RC or IL-17RC/IL-17RA with a threshold level of binding activity, and (2) antibodies do not significantly cross-react with polypeptides related to IL-17RC or IL-17RC/IL-17RA.

[232] With regard to the first characteristic, antibodies specifically bind if they bind to a IL-17RC or IL-17RC/IL-17RA polypeptide, peptide or epitope with a binding affinity (K_a) of $10^6 M^{-1}$ or greater, preferably $10^7 M^{-1}$ or greater, more preferably $10^8 M^{-1}$ or greater, and most preferably $10^9 M^{-1}$ or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci. 51*:660 (1949)). With regard to the second characteristic, antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect IL-17RC or IL-17RC/IL-17RA, but not presently known

polypeptides using a standard Western blot analysis. Examples of known related polypeptides include known cytokine receptors.

[233] Anti-IL-17RC or IL-17RC/IL-17RA antibodies can be produced using antigenic IL-17RC or IL-17RC/IL-17RA epitope-bearing peptides and polypeptides. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, or between 15 to about 30 amino acids contained within SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5 or another amino acid sequence disclosed herein. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that bind with IL-17RC or IL-17RC/IL-17RA. It is desirable that the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues, while hydrophobic residues are typically avoided). Moreover, amino acid sequences containing proline residues may be also be desirable for antibody production.

[234] As an illustration, potential antigenic sites in IL-17RC were identified using the Jameson-Wolf method, Jameson and Wolf, *CABIOS 4*:181, (1988), as implemented by the PROTEAN program (version 3.14) of LASERGENE (DNASTAR; Madison, WI). Default parameters were used in this analysis.

[235] The Jameson-Wolf method predicts potential antigenic determinants by combining six major subroutines for protein structural prediction. Briefly, the Hopp-Woods method, Hopp *et al., Proc. Nat'l Acad. Sci. USA 78*:3824 (1981), was first used to identify amino acid sequences representing areas of greatest local hydrophilicity (parameter: seven residues averaged). In the second step, Emini's method, Emini *et al., J. Virology 55*:836 (1985), was used to calculate surface probabilities (parameter: surface decision threshold (0.6) = 1). Third, the Karplus-Schultz method, Karplus and Schultz, *Naturwissenschaften 72*:212 (1985), was used to predict backbone chain flexibility (parameter: flexibility threshold (0.2) = 1). In the fourth and fifth steps of the analysis, secondary structure predictions were applied to the data using the methods of Chou-Fasman, Chou, "Prediction of Protein Structural Classes from Amino Acid Composition," in *Prediction of Protein Structure and the Principles of Protein Conformation*, Fasman (ed.), pages 549-586 (Plenum Press 1990), and Garnier-Robson, Garnier *et al., J. Mol. Biol. 120*:97 (1978) (Chou-Fasman parameters: conformation table = 64 proteins; α region threshold = 103; β region threshold = 105; Garnier-Robson parameters: α and β decision constants = 0). In the sixth subroutine, flexibility parameters and hydropathy/solvent accessibility factors were combined to determine a surface contour value, designated as the "antigenic index." Finally, a peak broadening function was applied to the antigenic index, which broadens major surface peaks by adding 20, 40, 60, or 80% of the respective peak value to account for additional free energy derived from the mobility of surface regions relative to interior

regions. This calculation was not applied, however, to any major peak that resides in a helical region, since helical regions tend to be less flexible. Hopp/Woods hydrophilicity profiles can be used to determine regions that have the most antigenic potential within SEQ ID NO:3 (Hopp et al., Proc. Natl. Acad. Sci.78:3824-3828, 1981; Hopp, J. Immun. Meth. 88:1-18, 1986 and Triquier et al., Protein Engineering 11:153-169, 1998). The profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues were ignored. Moreover, IL-17RC antigenic epitopes within SEQ ID NO:3 as predicted by a Jameson-Wolf plot, e.g., using DNASTAR Protean program (DNASTAR, Inc., Madison, WI) serve as preferred antigenic epitopes, and can be determined by one of skill in the art. Such antigenic epitopes include (1) amino acid residue 73 to amino acid residue 82 of SEQ ID NO:3; (2) amino acid residue 95 to amino acid residue 104 of SEQ ID NO:3; (3) amino acid residue 111 to amino acid residue 119 of SEQ ID NO:3; (4) amino acid residue 179 to amino acid residue 186 of SEQ ID NO:3; (5) amino acid residue 200 to amino acid residue 205 of SEQ ID NO:3; (6) amino acid residue 229 to amino acid residue 236 of SEQ ID NO:3; (7) amino acid residue 264 to amino acid residue 268 of SEQ ID NO:3; and (8) amino acid residue 275 to amino acid residue 281 of SEQ ID NO:3. The present invention contemplates the use of any one of antigenic peptides X to Y to generate antibodies to IL-17RC or as a tool to screen or identify neutralizing monoclonal antibodies of the present invention. The present invention also contemplates polypeptides comprising at least one of antigenic peptides X to Y. The present invention contemplates the use of any antigenic peptides or epitopes described herein to generate antibodies to IL-17RC, as well as to identify and screen anti-IL-17RC monoclonal antibodies that are neutralizing, and that may bind, block, inhibit, reduce, antagonize or neutralize the activity of IL-17F and IL-17A (individually or together).

[236] Moreover, suitable antigens also include the IL-17RC or IL-17RC/IL-17RA polypeptides comprising a IL-17RC or IL-17RC/IL-17RA cytokine binding, or extracellular domain disclosed above in combination with another cytokine extracellular domain, such as a class I or II cytokine receptor domain, such as those that may form soluble IL-17RC or IL-17RC/IL-17RA heterodimeric or multimeric polypeptides, and the like.

[237] Polyclonal antibodies to recombinant IL-17RC or IL-17RC/IL-17RA protein or to IL-17RC or IL-17RC/IL-17RA isolated from natural sources can be prepared using methods well-known to those of skill in the art. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.), pages 1-5 (Humana Press 1992), and Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995). The immunogenicity of a IL-17RC or IL-17RC/IL-17RA polypeptide can be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion

polypeptides, such as fusions of IL-17RC or IL-17RC/IL-17RA or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like," such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

[238] Although polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep, an anti-IL-17RC or IL-17RC/IL-17RA antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising diagnostically and therapeutically useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465, and in Losman *et al., Int. J. Cancer 46*:310 (1990).

[239] Alternatively, monoclonal anti-IL-17RC or IL-17RC/IL-17RA antibodies can be generated. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler *et al., Nature 256*:495 (1975), Coligan *et al.* (eds.), *Current Protocols in Immunology, Vol. 1*, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"], Picksley *et al.*, "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 93 (Oxford University Press 1995)).

[240] Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising a IL-17RC or IL-17RC/IL-17RA gene product, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

[241] In addition, an anti-IL-17RC or IL-17RC/IL-17RA antibody of the present invention may be derived from a human monoclonal antibody. Human monoclonal antibodies are obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green *et al., Nature Genet. 7*:13 (1994), Lonberg *et al., Nature 368*:856 (1994), and Taylor *et al., Int. Immun. 6*:579 (1994).

[242] Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography

with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.,* "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology, Vol. 10,* pages 79-104 (The Humana Press, Inc. 1992)).

[243] For particular uses, it may be desirable to prepare fragments of anti-IL-17RC or IL-17RC/IL-17RA antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No. 4,331,647, Nisonoff *et al., Arch Biochem. Biophys. 89*:230 (1960), Porter, *Biochem. J. 73*:119 (1959), Edelman *et al.*, in *Methods in Enzymology Vol. 1*, page 422 (Academic Press 1967), and by Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

[244] Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[245] For example, Fv fragments comprise an association of V_H and V_L chains. This association can be noncovalent, as described by Inbar *et al., Proc. Nat'l Acad. Sci. USA 69*:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (see, for example, Sandhu, *Crit. Rev. Biotech. 12*:437 (1992)).

[246] The Fv fragments may comprise V_H and V_L chains which are connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector which is subsequently introduced into a host cell, such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow *et al., Methods: A Companion to Methods in Enzymology 2*:97 (1991) (also see, Bird *et al., Science 242*:423 (1988), Ladner *et al.*, U.S. Patent No. 4,946,778, Pack *et al., Bio/Technology 11*:1271 (1993), and Sandhu, *supra*).

[247] As an illustration, a scFV can be obtained by exposing lymphocytes to IL-17RC or IL-17RC/IL-17RA polypeptide *in vitro*, and selecting antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled IL-17RC or IL-17RC/IL-17RA protein or peptide). Genes encoding polypeptides having potential IL-17RC or IL-17RC/IL-17RA polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223,409, Ladner *et al.*, U.S. Patent No. 4,946,778, Ladner *et al.*, U.S. Patent No. 5,403,484, Ladner *et al.*, U.S. Patent No. 5,571,698, and Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press, Inc. 1996)) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the IL-17RC or IL-17RC/IL-17RA sequences disclosed herein to identify proteins which bind to IL-17RC or IL-17RC/IL-17RA.

[248] Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick *et al., Methods: A Companion to Methods in Enzymology 2*:106 (1991), Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995), and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995)).

[249] Alternatively, an anti-IL-17RC or IL-17RC/IL-17RA antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin

variable domains are described, for example, by Orlandi *et al., Proc. Nat'l Acad. Sci. USA 86*:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al., Nature 321*:522 (1986), Carter *et al., Proc. Nat'l Acad. Sci. USA 89*:4285 (1992), Sandhu, *Crit. Rev. Biotech. 12*:437 (1992), Singer *et al., J. Immun. 150*:2844 (1993), Sudhir (ed.), *Antibody Engineering Protocols* (Humana Press, Inc. 1995), Kelley, "Engineering Therapeutic Antibodies," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Patent No. 5,693,762 (1997).

[250] Moreover, anti-IL-17RC or IL-17RC/IL-17RA antibodies or antibody fragments of the present invention can be PEGylated using methods in the art and described herein.

[251] Polyclonal anti-idiotype antibodies can be prepared by immunizing animals with anti-IL-17RC or IL-17RC/IL-17RA antibodies or antibody fragments, using standard techniques. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Methods In Molecular Biology: Immunochemical Protocols*, Manson (ed.), pages 1-12 (Humana Press 1992). Also, see Coligan at pages 2.4.1-2.4.7. Alternatively, monoclonal anti-idiotype antibodies can be prepared using anti-IL-17RC or IL-17RC/IL-17RA antibodies or antibody fragments as immunogens with the techniques, described above. As another alternative, humanized anti-idiotype antibodies or subhuman primate anti-idiotype antibodies can be prepared using the above-described techniques. Methods for producing anti-idiotype antibodies are described, for example, by Irie, U.S. Patent No. 5,208,146, Greene, *et. al.*, U.S. Patent No. 5,637,677, and Varthakavi and Minocha, *J. Gen. Virol. 77*:1875 (1996).

[252] An anti-IL-17RC or IL-17RC/IL-17RA antibody can be conjugated with a detectable label to form an anti-IL-17RC or IL-17RC/IL-17RA immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

[253] The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are ^{3}H, ^{125}I, ^{131}I, ^{35}S and ^{14}C.

[254] Anti-IL-17RC or IL-17RC/IL-17RA immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

[255] Alternatively, anti-IL-17RC or IL-17RC/IL-17RA immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds

include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

[256] Similarly, a bioluminescent compound can be used to label anti-IL-17RC or IL-17RC/IL-17RA immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

[257] Alternatively, anti-IL-17RC or IL-17RC/IL-17RA immunoconjugates can be detectably labeled by linking an anti-IL-17RC or IL-17RC/IL-17RA antibody component to an enzyme. When the anti-IL-17RC or IL-17RC/IL-17RA-enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

[258] Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to anti-IL-17RC or IL-17RC/IL-17RA antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy *et al., Clin. Chim. Acta 70*:1 (1976), Schurs *et al., Clin. Chim. Acta 81*:1 (1977), Shih *et al., Int'l J. Cancer 46*:1101 (1990), Stein *et al., Cancer Res. 50:*1330 (1990), and Coligan, *supra.*

[259] Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-IL-17RC or IL-17RC/IL-17RA antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek *et al.* (eds.), "Avidin-Biotin Technology," *Methods In Enzymology, Vol. 184* (Academic Press 1990), and Bayer *et al.*, "Immunochemical Applications of Avidin-Biotin Technology," in *Methods In Molecular Biology, Vol. 10*, Manson (ed.), pages 149-162 (The Humana Press, Inc. 1992).

[260] Methods for performing immunoassays are well-established. See, for example, Cook and Self, "Monoclonal Antibodies in Diagnostic Immunoassays," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 180-208, (Cambridge University Press, 1995), Perry, "The Role of Monoclonal Antibodies in the Advancement of Immunoassay Technology," in *Monoclonal Antibodies: Principles and Applications*, Birch and Lennox (eds.), pages 107-120 (Wiley-Liss, Inc. 1995), and Diamandis, *Immunoassay* (Academic Press, Inc. 1996).

[261] The present invention also contemplates kits for performing an immunological diagnostic assay for IL-17RC or IL-17RC/IL-17RA gene expression. Such kits comprise at least one

container comprising an anti-IL-17RC or IL-17RC/IL-17RA antibody, or antibody fragment. A kit may also comprise a second container comprising one or more reagents capable of indicating the presence of IL-17RC or IL-17RC/IL-17RA antibody or antibody fragments. Examples of such indicator reagents include detectable labels such as a radioactive label, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label, colloidal gold, and the like. A kit may also comprise a means for conveying to the user that IL-17RC or IL-17RC/IL-17RA antibodies or antibody fragments are used to detect IL-17RC or IL-17RC/IL-17RA protein. For example, written instructions may state that the enclosed antibody or antibody fragment can be used to detect IL-17RC or IL-17RC/IL-17RA. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

I) Therapeutic Uses of the IL-17RC or IL-17RC/IL-17RA Polypeptides of the Invention

[262] Amino acid sequences having soluble IL-17RC or IL-17RC/IL-17RA activity can be used to modulate the immune system by binding ligands IL-17A and IL-17F (either singly or together), and thus, preventing the binding of these ligands with endogenous IL-17RC and/or IL-17RA receptor. Such antagonists, such as soluble IL-17RC or IL-17RC/IL-17RA, can also be used to modulate the immune system by inhibiting the binding of IL-17A and/or IL-17F with the endogenous IL-17RC and/or IL-17RA receptor. Accordingly, the present invention includes the use of proteins, polypeptides, and peptides having IL-17RC or IL-17RC/IL-17RA activity (such as soluble IL-17RC or IL-17RC/IL-17RA polypeptides, IL-17RC or IL-17RA polypeptide fragments, IL-17RC or IL-17RC/IL-17RA analogs, and IL-17RC or IL-17RC/IL-17RA fusion proteins) to a subject which lacks an adequate amount of this polypeptide, or which produces an excess of IL-17A and/or IL-17F. The polypeptides of the present invention (*e.g.,* soluble IL-17RC and/or IL-17RC/IL-17RA) can be also used to treat a subject which produces an excess of either IL-17A, IL-17F, IL-17RA or IL-17RC. Suitable subjects include mammals, such as humans. For example, such soluble polypeptides are useful in binding, blocking, inhibiting, reducing, antagonizing or neutralizing IL-17A and IL-17F (either singly or together), in the treatment of inflammation and inflammatory dieases such as psoriasis, psoriatic arthritis, rheumatoid arthritis, endotoxemia, IBD, IBS, colitis, asthma, allograft rejection, immune mediated renal diseases, hepatobiliary diseases, multiple sclerosis, atherosclerosis, promotion of tumor growth, or degenerative joint disease and other inflammatory conditions disclosed herein.

[263] Within preferred embodiments, the soluble receptor comprisies IL-17RC (SEQ ID NO:3) and is a monomer, homodimer, heterodimer, or multimer that binds to, blocks, inhibits, reduces, antagonizes or neutralizes IL-17F and IL-17A (individually or together) *in vivo*. Antibodies and binding polypeptides to such IL-17RC monomer, homodimer, heterodimer, or multimers also

serve as antagonists of IL-17RC activity, and as IL-17A and IL-17F antagonists (singly or together), as described herein.

[264] Within other preferred embodiments, the soluble receptor comprises portions both IL-17RC and IL-17RA. One such preferred embodiment is Variant 1454 (SEQ ID NOs: 157 and 158) which includes exons 1-6 of human IL-17RA and 8-16 of human IL-17RCx1, fused to Fc5 (SEQ ID NOs: 179 and 180). Variant 1454 also has the native signal peptide from human IL-17RA. Fc10, or any equivalent known in the art, may also be used in place of Fc5.

[265] In addition, we have described herein that both polyclonal and monoclonal neutralizing anti-IL-17F antibodies bind to, block, inhibit, reduce, antagonize or neutralize IL-17F and IL-17A activity in cell based neutralization assays. Analysis of the tissue distribution of the mRNA corresponding IL-17RC cDNA showed that mRNA the *IL-17RC* gene is strongly expressed in thyroid, adrenal gland, prostate, and liver tissues, and expressed to a lesser extent in heart, small intestine, stomach, and trachea tissues. In particular, IL-17RC is consistently expressed in non-T cell peripheral blood cell lines, including monocytes, B-cells, and cells of the myeloid lineage. Also, IL-17RC mRNA is reliably expressed in cell lines derived from skin. Other cell lines that express IL-17RC are all 5 of the large intestine cell lines that were present on the array. In contrast, there is little or no expression in brain, placenta, lung, skeletal muscle, kidney, pancreas, spleen, thymus, testis, ovary, colon, peripheral blood leukocytes, spinal cord, lymph node, and bone marrow. The ligand to which IL-17RC binds (IL-17F and/or IL-17A) is implicated in inducing inflammatory response and contributing to inflammatory diseases, primarily via its ability to enhance production of inflammatory mediators, including IL-1b, IL-6 and TNF-a, as well as those mediators that are involved in the proliferation, maturation and chemotaxis of neutrophils (reviewed in Witowski et al. Cell. Mol. Life Sci. 61:567-579 [2004]).

[266] Thus, particular embodiments of the present invention are directed toward use of soluble IL-17RC and soluble IL-17RC/IL-17RA polypeptides as antagonists in inflammatory and immune diseases or conditions such as psoriasis, psoriatic arthritis, atopic dermatitis, inflammatory skin conditions, rheumatoid arthritis, IBD, IBS, Crohn's Disease, diverticulosis, asthma, pancreatitis, type I diabetes (IDDM), pancreatic cancer, pancreatitis, Graves Disease, colon and intestinal cancer, autoimmune disease, sepsis, organ or bone marrow transplant; inflammation due to endotoxemia, trauma, sugery or infection; amyloidosis; splenomegaly; graft versus host disease; and where inhibition of inflammation, immune suppression, reduction of proliferation of hematopoietic, immune, inflammatory or lymphoid cells, macrophages, T-cells (including Th1 and Th2 cells), suppression of immune response to a pathogen or antigen, or other instances where inhibition of IL-17F and/or IL-17A is desired.

[267] Moreover, soluble IL-17RC and soluble IL-17RC/IL-17RA polypeptides are useful to:

[268] (1) Block, inhibit, reduce, antagonize or neutralize signaling via IL-17RA or IL-17RC in the treatment of acute inflammation, inflammation as a result of trauma, tissue injury, surgery, sepsis or infection, and chronic inflammatory diseases such as asthma, inflammatory bowel disease (IBD), IBS, chronic colitis, splenomegaly, rheumatoid arthritis, recurrent acute inflammatory episodes (e.g., tuberculosis), and treatment of amyloidosis, and atherosclerosis, Castleman's Disease, asthma, and other diseases associated with the induction of acute-phase response.

[269] (2) Block, inhibit, reduce, antagonize or neutralize signaling IL-17RA or IL-17RC in the treatment of autoimmune diseases such as IDDM, multiple sclerosis (MS), systemic Lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis, IBS and IBD to prevent or inhibit signaling in immune cells (e.g. lymphocytes, monocytes, leukocytes). Blocking, inhibiting, reducing, or antagonizing signaling via IL-17RC and/or IL-17RA, using the polypeptides of the present invention, may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit. IL-17RC and/or IL-17RA may serve as a target for treatment of cancer where an antagonist of the present invention inhibits cancer growth and targets immune-mediated killing. (Holliger P, and Hoogenboom, H: Nature Biotech. 16: 1015-1016, 1998). Soluble polypeptides of the present invention may also be useful to treat nephropathies such as glomerulosclerosis, membranous neuropathy, amyloidosis (which also affects the kidney among other tissues), renal arteriosclerosis, glomerulonephritis of various origins, fibroproliferative diseases of the kidney, as well as kidney dysfunction associated with SLE, IDDM, type II diabetes (NIDDM), renal tumors and other diseases.

[270] (3) Agonize, enhance, increase or initiate signaling via IL-17RA or IL-17RC in the treatment of autoimmune diseases such as IDDM, MS, SLE, myasthenia gravis, rheumatoid arthritis, IBS and IBD. The soluble polypeptides of the present invention may signal lymphocytes or other immune cells to differentiate, alter proliferation, or change production of cytokines or cell surface proteins that ameliorate autoimmunity. Specifically, modulation of a T-helper cell response to an alternate pattern of cytokine secretion may deviate an autoimmune response to ameliorate disease (Smith JA et al., J. Immunol. 160:4841-4849, 1998). Similarly, agonistic soluble polypeptides may be used to signal, deplete and deviate immune cells involved in asthma, allergy and atopoic disease. Signaling via IL-17RC and/or IL-17RA may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit.

[271] Soluble IL-17RC or IL-17RC/IL-17RA polypeptides described herein can be used to bind, block, inhibit, reduce, antagonize or neutralize IL-17F or IL-17A activity, either singly or together, in the treatment of autoimmune disease, atopic disease, NIDDM, pancreatitis and kidney dysfunction as described above. A soluble form of IL-17RC or IL-17RC/IL-17RA may be used to promote an antibody response mediated by Th cells and/or to promote the production of IL-4 or other cytokines by lymphocytes or other immune cells.

[272] The soluble polypeptides of the present invention are useful as antagonists of IL-17A and/or IL-17F. Such antagonistic effects can be achieved by direct neutralization or binding of IL-17A or IL-17F. In addition to antagonistic uses, the soluble receptors of the present invention can bind IL-17F or IL-17A and act as carrier proteins for the ligand, in order to transport it to different tissues, organs, and cells within the body. As such, the soluble receptors of the present invention can be fused or coupled to molecules, polypeptides or chemical moieties that direct the soluble-receptor-Ligand complex to a specific site, such as a tissue, specific immune cell, or tumor. For example, in acute infection or some cancers, benefit may result from induction of inflammation and local acute phase response proteins by the action of IL-17F. Thus, the soluble receptors of the present invention can be used to specifically direct the action of IL-17A or IL-17F. See, Cosman, D. Cytokine 5: 95-106, 1993; and Fernandez-Botran, R. Exp. Opin. Invest. Drugs 9:497-513, 2000.

[273] Inflammation is a protective response by an organism to fend off an invading agent. Inflammation is a cascading event that involves many cellular and humoral mediators. On one hand, suppression of inflammatory responses can leave a host immunocompromised; however, if left unchecked, inflammation can lead to serious complications including chronic inflammatory diseases (e.g., psoriasis, arthritis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and the like), septic shock and multiple organ failure. Importantly, these diverse disease states share common inflammatory mediators. The collective diseases that are characterized by inflammation have a large impact on human morbidity and mortality. Therefore it is clear that anti-inflammatory proteins, such as the soluble polypeptides of the present invention could have crucial therapeutic potential for a vast number of human and animal diseases, from asthma and allergy to autoimmunity and septic shock.

1. Arthritis

[274] Arthritis, including osteoarthritis, rheumatoid arthritis, arthritic joints as a result of injury, and the like, are common inflammatory conditions which would benefit from the therapeutic use of anti-inflammatory proteins, such as the soluble polypeptides of the present invention. For example, rheumatoid arthritis (RA) is a systemic disease that affects the entire body and is one of the most common forms of arthritis. It is characterized by the inflammation of the membrane lining the joint, which causes pain, stiffness, warmth, redness and swelling. Inflammatory cells release enzymes that may digest bone and cartilage. As a result of rheumatoid arthritis, the inflamed joint lining, the synovium, can invade and damage bone and cartilage leading to joint deterioration and severe pain amongst other physiologic effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement.

[275] Rheumatoid arthritis (RA) is an immune-mediated disease particularly characterized by inflammation and subsequent tissue damage leading to severe disability and increased mortality. A variety of cytokines are produced locally in the rheumatoid joints. Numerous studies have demonstrated that IL-1 and TNF-alpha, two prototypic pro-inflammatory cytokines, play an important

role in the mechanisms involved in synovial inflammation and in progressive joint destruction. Indeed, the administration of TNF-alpha and IL-1 inhibitors in patients with RA has led to a dramatic improvement of clinical and biological signs of inflammation and a reduction of radiological signs of bone erosion and cartilage destruction. However, despite these encouraging results, a significant percentage of patients do not respond to these agents, suggesting that other mediators are also involved in the pathophysiology of arthritis (Gabay, Expert. Opin. Biol. Ther. 2(2):135-149, 2002). One of those mediators could be IL-17A or IL-17F, and as such a molecule that binds or inhibits IL-17F or IL-17A activity, such as soluble IL-17RC or IL-17RC/IL-17RA, could serve as a valuable therapeutic to reduce inflammation in rheumatoid arthritis, and other arthritic diseases.

[276] There are several animal models for rheumatoid arthritis known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human rheumatoid arthritis. Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. The CIA model is a well-known model in mice that depends on both an immune response, and an inflammatory response, in order to occur. The immune response comprises the interaction of B-cells and CD4+ T-cells in response to collagen, which is given as antigen, and leads to the production of anti-collagen antibodies. The inflammatory phase is the result of tissue responses from mediators of inflammation, as a consequence of some of these antibodies cross-reacting to the mouse's native collagen and activating the complement cascade. An advantage in using the CIA model is that the basic mechanisms of pathogenesis are known. The relevant T-cell and B-cell epitopes on type II collagen have been identified, and various immunological (e.g., delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (e.g., cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediated arthritis have been determined, and can thus be used to assess test compound efficacy in the CIA model (Wooley, Curr. Opin. Rheum. 3:407-20, 1999; Williams et al., Immunol. 89:9784-788, 1992; Myers et al., Life Sci. 61:1861-78, 1997; and Wang et al., Immunol. 92:8955-959, 1995).

[277] One group has shown that an anti-mouse IL-17 antibody reduces symptoms in a mouse CIA-model relative to control mice, thus showing conceptually that the soluble polypeptides of the present invention would be beneficial in treating human disease. The administration of a single mouse-IL-17-specific rat antisera reduced the symptoms of arthritis in the animals when introduced prophylactically or after symptoms of arthritis were already present in the model (Lubberts et al, Arthritis Rheum. 50:650-9, 2004). Therefore, IL-17RC-Fc or IL-17RC/IL-17RA-Fc can be used to neutralize IL-17A and/or IL-17F in the treatment of specific human diseases such as arthritis, psoriasis, psoriatic arthritis, endotoxemia, inflammatory bowel disease (IBD), IBS, colitis, and other inflammatory conditions disclosed herein.

[278] The administration of the soluble polypeptides of the present invention, such as IL-17RC-Fc or other IL-17RC/IL-17RA soluble and fusion proteins to these CIA model mice is used to evaluate their use as an antagonist to IL-17F and IL-17A to ameliorate symptoms and alter the course of disease. Moreover, results showing inhibition or neutralization of IL-17F and/or IL-17A by the soluble polypeptides of the present invention would provide proof of concept that other IL-17A or Il-17F antagonists can also be used to ameliorate symptoms and alter the course of disease. Furthermore, since IL-17A and/or IL-17F induces production of IL-1b and TNF-a, both of which are implicated in the pathogenesis and progression of rheumatoid arthritis, the systemic or local administration of these soluble polypeptides can potentially suppress the inflammatory response in RA. By way of example and without limitation, the injection of 10 - 200 ug IL-17RC-Fc per mouse (one to seven times a week for up to but not limited to 4 weeks via s.c., i.p., or i.m route of administration) can significantly reduce the disease score (paw score, incident of inflammation, or disease). Depending on the initiation of IL-17RC-Fc administration (e.g. prior to or at the time of collagen immunization, or at any time point following the second collagen immunization, including those time points at which the disease has already progressed), IL-17RC can be efficacious in preventing rheumatoid arthritis, as well as preventing its progression. Other potential therapeutics include IL-17RC/IL-17RA polypeptides, and the like.

2. Endotoxemia

[279] Endotoxemia is a severe condition commonly resulting from infectious agents such as bacteria and other infectious disease agents, sepsis, toxic shock syndrome, or in immunocompromised patients subjected to opportunistic infections, and the like. Therapeutically useful of anti-inflammatory proteins, such as the soluble polypeptides of the present invention could aid in preventing and treating endotoxemia in humans and animals. These soluble polypeptides could serve as a valuable therapeutic to reduce inflammation and pathological effects in endotoxemia.

[280] Lipopolysaccharide (LPS) induced endotoxemia engages many of the proinflammatory mediators that produce pathological effects in the infectious diseases and LPS induced endotoxemia in rodents is a widely used and acceptable model for studying the pharmacological effects of potential pro-inflammatory or immunomodulating agents. LPS, produced in gram-negative bacteria, is a major causative agent in the pathogenesis of septic shock (Glausner et al., Lancet 338:732, 1991). A shock-like state can indeed be induced experimentally by a single injection of LPS into animals. Molecules produced by cells responding to LPS can target pathogens directly or indirectly. Although these biological responses protect the host against invading pathogens, they may also cause harm. Thus, massive stimulation of innate immunity, occurring as a result of severe Gram-negative bacterial infection, leads to excess production of cytokines and other molecules, and the development of a fatal syndrome, septic shock syndrome, which is characterized by fever,

hypotension, disseminated intravascular coagulation, and multiple organ failure (Dumitru et al. Cell 103:1071-1083, 2000).

[281] These toxic effects of LPS are mostly related to macrophage activation leading to the release of multiple inflammatory mediators. Among these mediators, TNF appears to play a crucial role, as indicated by the prevention of LPS toxicity by the administration of neutralizing anti-TNF antibodies (Beutler et al., Science 229:869, 1985). It is well established that 1ug injection of *E. coli* LPS into a C57Bl/6 mouse will result in significant increases in circulating IL-6, TNF-alpha, IL-1, and acute phase proteins (for example, SAA) approximately 2 hours post injection. The toxicity of LPS appears to be mediated by these cytokines as passive immunization against these mediators can result in decreased mortality (Beutler et al., Science 229:869, 1985). The potential immunointervention strategies for the prevention and/or treatment of septic shock include anti-TNF mAb, IL-1 receptor antagonist, LIF, IL-10, and G-CSF.

[282] The administration of the soluble polypeptides of the present invention to these LPS-induced model may be used to to evaluate the use of IL-17RC or IL=17RC/IL-17RA to ameliorate symptoms and alter the course of LPS-induced disease. Moreover, results showing inhibition of IL-17F or IL-17A by these ssoluble polypeptides would provide proof of concept that other such antagonists can also be used to ameliorate symptoms in the LPS-induced model and alter the course of disease. The model will show induction of IL-17F by LPS injection and the potential treatment of disease by the soluble polypeptides. Since LPS induces the production of pro-inflammatory factors possibly contributing to the pathology of endotoxemia, the neutralization of IL-17F activity or other pro- inflammatory factors by an antagonist soluble polyepeptide can be used to reduce the symptoms of endotoxemia, such as seen in endotoxic shock.

3. Inflammatory Bowel Disease IBD

[283] In the United States approximately 500,000 people suffer from Inflammatory Bowel Disease (IBD) which can affect either colon and rectum (Ulcerative colitis) or both, small and large intestine (Crohn's Disease). The pathogenesis of these diseases is unclear, but they involve chronic inflammation of the affected tissues. The soluble polypeptides of the present invention could serve as a valuable therapeutic to reduce inflammation and pathological effects in IBD, UC and related diseases.

[284] Ulcerative colitis (UC) is an inflammatory disease of the large intestine, commonly called the colon, characterized by inflammation and ulceration of the mucosa or innermost lining of the colon. This inflammation causes the colon to empty frequently, resulting in diarrhea. Symptoms include loosening of the stool and associated abdominal cramping, fever and weight loss. Although the exact cause of UC is unknown, recent research suggests that the body's natural defenses are operating against proteins in the body which the body thinks are foreign (an "autoimmune reaction"). Perhaps because they resemble bacterial proteins in the gut, these proteins may either instigate or

stimulate the inflammatory process that begins to destroy the lining of the colon. As the lining of the colon is destroyed, ulcers form releasing mucus, pus and blood. The disease usually begins in the rectal area and may eventually extend through the entire large bowel. Repeated episodes of inflammation lead to thickening of the wall of the intestine and rectum with scar tissue. Death of colon tissue or sepsis may occur with severe disease. The symptoms of ulcerative colitis vary in severity and their onset may be gradual or sudden. Attacks may be provoked by many factors, including respiratory infections or stress.

[285] Although there is currently no cure for UC available, treatments are focused on suppressing the abnormal inflammatory process in the colon lining. Treatments including corticosteroids immunosuppressives (eg. azathioprine, mercaptopurine, and methotrexate) and aminosalicytates are available to treat the disease. However, the long-term use of immunosuppressives such as corticosteroids and azathioprine can result in serious side effects including thinning of bones, cataracts, infection, and liver and bone marrow effects. In the patients in whom current therapies are not successful, surgery is an option. The surgery involves the removal of the entire colon and the rectum.

[286] There are several animal models that can partially mimic chronic ulcerative colitis. The most widely used model is the 2,4,6-trinitrobenesulfonic acid/ethanol (TNBS) induced colitis model, which induces chronic inflammation and ulceration in the colon. When TNBS is introduced into the colon of susceptible mice via intra-rectal instillation, it induces T-cell mediated immune response in the colonic mucosa, in this case leading to a massive mucosal inflammation characterized by the dense infiltration of T-cells and macrophages throughout the entire wall of the large bowel. Moreover, this histopathologic picture is accompanies by the clinical picture of progressive weight loss (wasting), bloody diarrhea, rectal prolapse, and large bowel wall thickening (Neurath et al. Intern. Rev. Immunol. 19:51-62, 2000).

[287] Another colitis model uses dextran sulfate sodium (DSS), which induces an acute colitis manifested by bloody diarrhea, weight loss, shortening of the colon and mucosal ulceration with neutrophil infiltration. DSS-induced colitis is characterized histologically by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal crypt damage, and epithelial ulceration. These changes are thought to develop due to a toxic effect of DSS on the epithelium and by phagocytosis of lamina propria cells and production of TNF-alpha and IFN-gamma. Despite its common use, several issues regarding the mechanisms of DSS about the relevance to the human disease remain unresolved. DSS is regarded as a T cell-independent model because it is observed in T cell-deficient animals such as SCID mice.

[288] The administration of the soluble polypeptides of the present invention to these TNBS or DSS models can be used to evaluate their use to ameliorate symptoms and alter the course of gastrointestinal disease. Moreover, the results showing inhibition or neutralization of IL-17F and/or

IL-17A by these soluble polypeptides provide proof of concept that they (or similar molecules) can also be used to ameliorate symptoms in the colitis/IBD models and alter the course of disease.

4. Psoriasis

[289] Psoriasis is a chronic skin condition that affects more than seven million Americans. Psoriasis occurs when new skin cells grow abnormally, resulting in inflamed, swollen, and scaly patches of skin where the old skin has not shed quickly enough. Plaque psoriasis, the most common form, is characterized by inflamed patches of skin ("lesions") topped with silvery white scales. Psoriasis may be limited to a few plaques or involve moderate to extensive areas of skin, appearing most commonly on the scalp, knees, elbows and trunk. Although it is highly visible, psoriasis is not a contagious disease. The pathogenesis of the diseases involves chronic inflammation of the affected tissues. The soluble polypeptides of the present invention could serve as a valuable therapeutic to reduce inflammation and pathological effects in psoriasis, other inflammatory skin diseases, skin and mucosal allergies, and related diseases.

[290] Psoriasis is a T-cell mediated inflammatory disorder of the skin that can cause considerable discomfort. It is a disease for which there is no cure and affects people of all ages. Psoriasis affects approximately two percent of the populations of European and North America. Although individuals with mild psoriasis can often control their disease with topical agents, more than one million patients worldwide require ultraviolet or systemic immunosuppressive therapy. Unfortunately, the inconvenience and risks of ultraviolet radiation and the toxicities of many therapies limit their long-term use. Moreover, patients usually have recurrence of psoriasis, and in some cases rebound, shortly after stopping immunosuppressive therapy.

[291] The soluble polypeptides of the present invention may also be used within diagnostic systems for the detection of circulating levels of IL-17F or IL-17A, and in the detection of IL-17F or IL-17A associated with acute phase inflammatory response. Within a related embodiment, the soluble polypeptides of the present invention can be used to detect circulating or locally-acting IL-17F or IL-17A polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including inflammation or cancer. IL-17F is known to induce associated acute phase inflammatory response. Moreover, detection of acute phase proteins or molecules such as IL-17A or IL-17F can be indicative of a chronic inflammatory condition in certain disease states (e.g., asthma, psoriasis, rheumatoid arthritis, colitis, IBD, IBS). Detection of such conditions serves to aid in disease diagnosis as well as help a physician in choosing proper therapy.

[292] In addition to other disease models described herein, the activity of the soluble polypeptides of the present invention on inflammatory tissue derived from human psoriatic lesions can be measured *in vivo* using a severe combined immune deficient (SCID) mouse model. Several mouse models have been developed in which human cells are implanted into immunodeficient mice (collectively referred to as xenograft models); see, for example, Cattan AR, Douglas E, Leuk. Res.

18:513-22, 1994 and Flavell, DJ, Hematological Oncology 14:67-82, 1996. As an in vivo xenograft model for psoriasis, human psoriatic skin tissue is implanted into the SCID mouse model, and challenged with an appropriate antagonist. Moreover, other psoriasis animal models in ther art may be used to evaluate IL-17A and IL-17F antagonists, such as human psoriatic skin grafts implanted into AGR129 mouse model, and challenged with an appropriate antagonist (e.g., see, Boyman, O. et al., J. Exp. Med. Online publication #20031482, 2004, incorporated hereing by reference). The soluble polypeptides of the present invention that bind, block, inhibit, reduce, antagonize or neutralize the activity of IL-17F or both IL-17A and IL-17F are preferred antagonists, as well as other IL-17A and IL-17F antagonists can be used in this model. Similarly, tissues or cells derived from human colitis, IBD, IBS, arthritis, or other inflammatory lestions can be used in the SCID model to assess the anti-inflammatory properties of the IL-17A and IL-17F antagonists described herein.

[293] Therapies designed to abolish, retard, or reduce inflammation using the soluble polypeptides of the present invention can be tested by administration to SCID mice bearing human inflammatory tissue (e.g., psoriatic lesions and the like), or other models described herein. Efficacy of treatment is measured and statistically evaluated as increased anti-inflammatory effect within the treated population over time using methods well known in the art. Some exemplary methods include, but are not limited to measuring for example, in a psoriasis model, epidermal thickness, the number of inflammatory cells in the upper dermis, and the grades of parakeratosis. Such methods are known in the art and described herein. For example, see Zeigler, M. et al. Lab Invest 81:1253, 2001; Zollner, T. M. et al. J. Clin. Invest. 109:671, 2002; Yamanaka, N. et al. Microbio.l Immunol. 45:507, 2001; Raychaudhuri, S. P. et al. Br. J. Dermatol. 144:931, 2001; Boehncke, W. H et al. Arch. Dermatol. Res. 291:104, 1999; Boehncke, W. H et al.. J. Invest. Dermatol. 116:596, 2001; Nickoloff, B. J. et al. Am. J. Pathol. 146:580, 1995; Boehncke, W. H et al. J. Cutan. Pathol. 24:1, 1997; Sugai, J., M. et al. J. Dermatol. Sci. 17:85, 1998; and Villadsen L.S. et al. J. Clin. Invest. 112:1571, 2003. Inflammation may also be monitored over time using well-known methods such as flow cytometry (or PCR) to quantitate the number of inflammatory or lesional cells present in a sample, score (weight loss, diarrhea, rectal bleeding, colon length) for IBD, paw disease score and inflammation score for CIA RA model. For example, therapeutic strategies appropriate for testing in such a model include direct treatment using soluble IL-17RC or IL-17RC/IL-17RA, or other IL-17A and IL-17F antagonists (singly or together), or related conjugates or antagonists based on the disrupting interaction of IL-17RC and/or IL-17RA with their corresponding ligands.

[294] Psoriasis is a chronic inflammatory skin disease that is associated with hyperplastic epidermal keratinocytes and infiltrating mononuclear cells, including CD4+ memory T cells, neutrophils and macrophages (Christophers, Int. Arch. Allergy Immunol., 110:199, 1996). It is currently believed that environmental antigens play a significant role in initiating and contributing to the pathology of the disease. However, it is the loss of tolerance to self-antigens that is thought to

mediate the pathology of psoriasis. Dendritic cells and $CD4^+$ T cells are thought to play an important role in antigen presentation and recognition that mediate the immune response leading to the pathology. We have recently developed a model of psoriasis based on the CD4+CD45RB transfer model (Davenport et al., Internat. Immunopharmacol., 2:653-672). The soluble polypeptides of the present invention are administered to the mice. Inhibition of disease scores (skin lesions, inflammatory cytokines) indicates the effectiveness of those soluble polypeptides in psoriasis.

5. Atopic Dermatitis.

[295] AD is a common chronic inflammatory disease that is characterized by hyperactivated cytokines of the helper T cell subset 2 (Th2). Although the exact etiology of AD is unknown, multiple factors have been implicated, including hyperactive Th2 immune responses, autoimmunity, infection, allergens, and genetic predisposition. Key features of the disease include xerosis (dryness of the skin), pruritus (itchiness of the skin), conjunctivitis, inflammatory skin lesions, *Staphylococcus aureus* infection, elevated blood eosinophilia, elevation of serum IgE and IgG1, and chronic dermatitis with T cell, mast cell, macrophage and eosinophil infiltration. Colonization or infection with *S. aureus* has been recognized to exacerbate AD and perpetuate chronicity of this skin disease.

[296] AD is often found in patients with asthma and allergic rhinitis, and is frequently the initial manifestation of allergic disease. About 20% of the population in Western countries suffer from these allergic diseases, and the incidence of AD in developed countries is rising for unknown reasons. AD typically begins in childhood and can often persist through adolescence into adulthood. Current treatments for AD include topical corticosteroids, oral cyclosporin A, non-corticosteroid immunosuppressants such as tacrolimus (FK506 in ointment form), and interferon-gamma. Despite the variety of treatments for AD, many patients' symptoms do not improve, or they have adverse reactions to medications, requiring the search for other, more effective therapeutic agents. The soluble polypeptides of the present invention can be used to neutralize IL-17F and IL-17A in the treatment of specific human diseases such as atoptic dermatitis, inflammatory skin conditions, and other inflammatory conditions disclosed herein.

6. Asthma

[297] IL-17 plays an important role in allergen-induced T cell activation and neutrophilic influx in the airways. The receptor for IL-17 is expressed in the airways (Yao, et al. Immunity 3:811 (1995)) and IL-17 mediated neutrophil recruitment in allergic asthma is largely induced by the chemoattractant IL-8, GRO-□ and macrophage inflammatory protein-2 (MIP-2) produced by IL-17 stimulated human bronchial epithelial cells (HBECs) and human bronichial fibroblasts (Yao, et al. *J Immunol 155:5483* (1995)); Molet, et al. *J Allergy Clin Immunol 108:430 (2001)*). IL-17 also stimulates HBECs to release IL-6, a neutrophil-activating factor (Fossiez, et al, *J Exp Med 183:2593 (1996),* and Linden, et al. *Int Arch Allergy Immunol 126:179* (2001)) and has been shown to synergize

with TNF-□ to prolong the survival of human neutrophils in vitro (Laan, et al. *Eur Respir J 21:387* (2003)). Moreover, IL-17 is capable of amplifying the inflammatory responses in asthma by its ability to enhance the secretion of cytokines implicated in airway remodeling such as the profibrotic cytokines, IL-6 and IL-11 and inflammatory mediators granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) (Molet, et al. *J Allergy Clin Immunol 108:430 (2001)*).

[298] Clinical evidence shows that acute, severe exacerbations of asthma are associated with recruitment and activation of neutrophils in the airways, thus IL-17 is likely to play a significant role in asthma. Patients with mild asthma display a detectable increase in the local concentration of free, soluble IL-17A protein (Molet, et al. J Allergy Clin Immunol 108:430 (2001)) while healthy human volunteers with induced, severe airway inflammation due to the exposure to a swine confinement, display a pronounced increase in the concentration of free, soluble IL-17A protein in the bronchoalveolar space (Fossiez, et al, J Exp Med 183:2593 (1996), and Linden, et al. Int Arch Allergy Immunol 126:179 (2001)). Furthermore, IL-17 levels in sputum have correlated with individuals who have increased airway hyper-reactivity Barczyk, et al. Respir Med 97:726 (2003).

[299] In animal models of airway hyper-responsiveness, chronic inhalation of ovalbumin by sensitized mice resulted in bronchial eosinophilic inflammation and early induction of IL-17 mRNA expression in inflamed lung tissue, together with a bronchial neutrophilia Hellings, et al. Am J Respir Cell Mol Biol 28:42 (2003). Anti-IL-17 monoclonal antibodies strongly reduced bronchial neutrophilic influx but significantly enhanced IL-5 levels in both bronchoalveolar lavage fluid and serum, and aggravated allergen-induced bronchial eosinophilic influx, suggesting that IL-17A may be involved in determining the balance between neutrophil and eosinophil accumulation following antigen insult Id..

[300] Among the IL-17 family members, IL-17F is most closely related to IL-17A. The biological activities mediated by IL-17F are similar to those of IL-17A, where IL-17F stimulates production of IL-6, IL-8 and G-CSF Hurst, et al. J Immunol 169:443 (2002). IL-17F also induces production of IL-2, transforming growth factor (TGF)-□, and monocyte chemoattractant protein (MCP) in endothelial cells Starnes, et al. J Immunol 167:4137 (2001). Similarly, allergen challenge can increase local IL-17F in patients with allergic asthma Kawaguchi, et al. J Immunol 167:4430 (2001). Gene delivery of IL-17F in murine lung increases neutrophils in the bronchoalveolar space, while mucosal transfer of the IL-17F gene enhances the levels of Ag-induced pulmonary neutrophilia and airway responsiveness to methacholine Oda, et al. Am J Respir Crit Care Med 171:12 (2005).

[301] Apart from asthma, several chronic inflammatory airway diseases are characterized by neutrophil recruitment in the airways and IL-17 has been reported to play an important role in the pathogenesis of respiratory conditions such as chronic obstructive pulmonary disease (COPD), bacterial pneumonia and cystic fibrosis (Linden, et al. Eur Respir J 15:973 (2000), Ye, et al. Am J

Respir Cell Mol Biol 25:335 (2001), Rahman, et al. Clin Immunol 115:268 (2005)). An anti-IL-17A and/or anti-IL-17F therapeutic molecule could be demonstrated to be efficacious for chronic inflammatory airway disease in an in vitro model of inflammation. The ability of antagonists to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention to inhibit IL-17A or and/or IL-17F-induced cytokine and chemokine production from cultured HBECs or bronchial fibroblasts could be used as a measure of efficacy for such antagonists in the prevention of the production of inflammatory mediators directly resulting from IL-17A and/or F stimulation. If the addition of antagonists, such as the soluble polypeptides of the present invention, to IL-17F and/or IL-17A activity, markedly reduces the production and expression of inflammatory mediators, it would be expected to be efficacious in inflammatory aspects associated with chronic airway inflammation.

7. Irritable Bowel Syndrome ("IBS")

[302] Irritable bowel syndrome represents a disease characterized by abdominal pain or discomfort and an erratic bowel habit. IBS patients can be characterized into three main groups based on bowel habits: those with predominantly loose or frequent stools, those with predominantly hard or infrequent stools, and those with variable or normal stools (Talley et al., 2002). Altered intestinal motility, abnormalities in epithelial function, abnormal transit of stool and gas, and stress, may contribute to symptoms, while visceral hypersensitivity is a key feature in most patients. Genetic factors affecting pain-signaling and disturbances in central processing of afferent signals are postulated to predispose individuals to IBS following specific environmental exposures. Studies have also demonstrated that inflammatory responses in the colon may contribute to increased sensitivity of smooth muscle and enteric nerves and therefore perturb sensory-motor functions in the intestine (Collins et al., 2001). There is clinical overlap between IBS and IBD, with IBS-like symptoms frequently reported in patients before the diagnosis of IBD, and a higher than expected IBS symptoms in patients in remission from established IBD. Thus, these conditions may coexist with a higher than expected frequency, or may exist on a continuum, with IBS and IBD at different ends of the same spectrum. However, it should be noted that in most IBS patients, colonic biopsy specimens appear normal. Nevertheless, IBS significantly affects a very large number of individuals (U.S. prevalence in 2000, approximately 16 million individuals), resulting in a total cost burden of 1.7 billion dollars (year 2000). Thus, among the most prevalent and costly gastrointestinal diseases and disorders, IBS is second only to gastroesophageal reflux disease (GERD). Yet unlike GERD, treatment for IBS remains unsatisfactory (Talley et al., 2002; Farhadi et al., 21001; Collins et al., 2001), demonstrating that IBS clearly represents an unmet medical need.

[303] Converging disease models have been proposed that postulate an enhanced responsiveness of neural, immune or neuroimmune circuits in the central nervous system (CNS) or in the gut to central (psychosocial) or peripheral (tissue irritation, inflammation, infection) perturbations

of normal homeostasis (Talley et al., 2002). This enhanced responsiveness results in dysregulation of gut motility, epithelial function (immune, permeability), and visceral hypersensitivity, which in turn results in IBS symptoms.

[304] There may be a role for a number of different molecules in the pathogenesis of IBS including a role for molecules that stimulate neurons and those that are involved in initiation of inflammatory process. A number of our in-house molecules are known to be linked to possible activity on neurons due to their direct expression by neurons or expression of their receptors on neurons, including IL-17D, IL-17B and IL-31. Moreover, a number of IL-17 family members and related molecules have been associated with inflammation in the gut, including IL-17A, IL-17F, IL-23 and IL-31.

[305] Efficacy of inhibitors of these molecules could be tested *in vivo* in animal models of disease. Several animal models have been proposed that mimic key features of IBS and involve centrally targeted stimuli (stress) or peripherally targeted stimuli (infection, inflammation). Two examples of in vivo animal models that can be used to determine the effectiveness of inhibitors in the treatment of IBS are (i) models focusing on primary CNS-directed pathogeneisis of IBS (stress models), and (ii) models focusing on gut-directed inducers of stress (i.e. gut inflammation, infection or physical stress). It should be noted however, that events within the CNS or in the gastrointestinal (GI) tract do not occur in isolation and that symptoms of IBS most likely result from a complex interaction between signals from the CNS on the GI and vice versa.

J) Pharmaceutical Formulations

[306] For pharmaceutical use, the soluble polypeptides of the present invention are formulated for parenteral, particularly intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration will be by bolus injection, controlled release, e.g, using mini-pumps or other appropriate technology, or by infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a hematopoietic protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to provent protein loss on vial surfaces, etc. When utilizing such a combination therapy, the cytokines may be combined in a single formulation or may be administered in separate formulations. Methods of formulation are well known in the art and are disclosed, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton PA, 1990, which is incorporated herein by reference. Therapeutic doses will generally be in the range of 0.1 to 100 mg/kg of patient weight per day, preferably 0.5-20 mg/kg per day, with the exact dose determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level

of ordinary skill in the art. The proteins will commonly be administered over a period of up to 28 days following chemotherapy or bone-marrow transplant or until a platelet count of >20,000/mm^3, preferably >50,000/mm^3, is achieved. More commonly, the proteins will be administered over one week or less, often over a period of one to three days. In general, a therapeutically effective amount of the soluble polypeptides of the present invention in an amount sufficient to produce a clinically significant increase in the proliferation and/or differentiation of lymphoid or myeloid progenitor cells, which will be manifested as an increase in circulating levels of mature cells (e.g. platelets or neutrophils). Treatment of platelet disorders will thus be continued until a platelet count of at least 20,000/mm^3, preferably 50,000/mm^3, is reached. The soluble polypeptides of the present invention can also be administered in combination with other cytokines such as IL-3, -6 and -11; stem cell factor; erythropoietin; G-CSF and GM-CSF. Within regimens of combination therapy, daily doses of other cytokines will in general be: EPO, 150 U/kg; GM-CSF, 5-15 lg/kg; IL-3, 1-5 lg/kg; and G-CSF, 1-25 lg/kg. Combination therapy with EPO, for example, is indicated in anemic patients with low EPO levels.

[307] Generally, the dosage of administered soluble polypeptides will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of such soluble polypeptide which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate.

[308] Administration of the soluble polypeptides of the present invention to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

[309] Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev. 35*:199 (1999)). Dry or liquid particles comprising soluble IL-17RC or anti-IL-17RC antibodies can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (*e.g.,* Pettit and Gombotz, *TIBTECH 16*:343 (1998); Patton *et al., Adv. Drug Deliv. Rev. 35*:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at

therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of trascutaneous administration (Mitragotri *et al., Science 269*:850 (1995)). Transdermal delivery using electroporation provides another means to administer the soluble polypeptides of the present invention (Potts *et al., Pharm. Biotechnol. 10*:213 (1997)).

[310] A pharmaceutical composition comprising the soluble polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

[311] For purposes of therapy, the soluble polypeptides of the present invention and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a therapeutic molecule of the present invention and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response.

[312] A pharmaceutical composition comprising a soluble polypeptide of the present invention can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al., Pharm. Biotechnol. 10*:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)).

[313] Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in

composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

[314] Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

[315] The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

[316] Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al., Biol. Pharm. Bull. 16*:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al., Biol. Pharm. Bull. 20*:881 (1997)).

[317] Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example,

liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug Carrier Syst. 14*:287 (1997); Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Similarly, Wu *et al., Hepatology 27*:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al., Proc. Nat'l Acad. Sci. USA 94*:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

[318] In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)).

[319] Polypeptides and antibodies can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al., Infect. Immun. 31*:1099 (1981), Anderson *et al., Cancer Res. 50*:1853 (1990), and Cohen *et al., Biochim. Biophys. Acta 1063*:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

[320] Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem. 6*:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al., Science 281*:1161 (1998); Putney and Burke, *Nature Biotechnology 16*:153 (1998); Putney, *Curr. Opin. Chem. Biol. 2*:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for

intravenous administration of therapeutic proteins (see, for example, Gref *et al., Pharm. Biotechnol. 10*:167 (1997)).

[321] The present invention also contemplates chemically modified polypeptides having IL-17A and/or IL-17F binding activity such as IL-17RC or IL-17RC/IL-17RA monomeric, homodimeric, heterodimeric or multimeric soluble receptors, which a polypeptide is linked with a polymer, as discussed above.

[322] Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

[323] As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises one of the soluble polypeptides of the present invention. Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the composition is contraindicated in patients with known hypersensitivity to IL-17RC or IL-17RA.

[324] A pharmaceutical composition comprising soluble polypeptides of the present invention can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions, aerosols, droplets, topological solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al.,* Pharm. Biotechnol. 10:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)). Other solid forms include creams, pastes, other topological applications, and the like.

[325] Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in

composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

[326] Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

[327] The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

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[329] Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example,

liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, Crit. Rev. Ther. Drug Carrier Syst. 14:287 (1997); Murahashi *et al.*, Biol. Pharm. Bull. 20:259 (1997)). Similarly, Wu *et al.*, Hepatology 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al.*, Biol. Pharm. Bull. 20:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al.*, Proc. Nat'l Acad. Sci. USA 94:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

[330] In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al.*, Adv. Drug Deliv. Rev. 32:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al.*, Adv. Drug Deliv. Rev. 32:99 (1998)).

[331] The soluble polypeptides of the present invention can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al.*, Infect. Immun. 31:1099 (1981), Anderson *et al.*, Cancer Res. 50:1853 (1990), and Cohen *et al.*, Biochim. Biophys. Acta 1063:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al.*, Biochim. Biophys. Acta 1150:9 (1993)).

[332] Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, Bioconjugate Chem. 6:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al.*, Science 281:1161 (1998); Putney and Burke, Nature Biotechnology 16:153 (1998); Putney, Curr. Opin. Chem. Biol. 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for

intravenous administration of therapeutic proteins (see, for example, Gref *et al.,* Pharm. Biotechnol. 10:167 (1997)).

[333] Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

[334] The present invention contemplates compositions of the soluble polypeptides of the present invention, and methods and therapeutic uses comprising the same polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

K) Production of Transgenic Mice

[335] Transgenic mice can be engineered to over-express the either IL-17F, IL-17A, IL-17RA or the IL-17RC gene in all tissues or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess IL-17F, IL-17A, IL-17RA or IL-17RC. Transgenic mice that over-express any of these also provide model bioreactors for production of IL-17RA or IL-17RC, such as any of the soluble polypeptides of the present invention in milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in Overexpression and Knockout of Cytokines in Transgenic Mice, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), Strategies in Transgenic Animal Science (ASM Press 1995), and Abbud and Nilson, "Recombinant Protein Expression in Transgenic Mice," in Gene Expression Systems: Using Nature for the Art of Expression, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

[336] For example, a method for producing a transgenic mouse that expresses a IL-17RC gene can begin with adult, fertile males (studs) (B6C3f1, 2-8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2-8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4-5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2-4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8 IU/mouse of Pregnant Mare's Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) I.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) I.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection. Copulation is confirmed by the presence of a vaginal plug the morning following mating.

[337] Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinanalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten's W640 medium (described, for example, by Menino and O'Claray, Biol. Reprod. 77:159 (1986), and Dienhart and Downs, Zygote 4:129 (1996)) that has been incubated with 5% CO2, 5% O2, and 90% N2 at 37°C. The eggs are then stored in a 37°C/5% CO2 incubator until microinjection.

[338] Ten to twenty micrograms of plasmid DNA containing a IL-17RC encoding sequence is linearized, gel-purified, and resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the IL-17RC encoding sequences can encode a polypeptide comprising amino acid residues 21 to 452 of SEQ ID NO:2.

[339] Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO2-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

[340] Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO2 incubator.

[341] The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

[342] With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day's injection are transferred into the recipient. The swollen ampulla is located and holding the oviduct between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

[343] The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the

skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

[344] The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

[345] Genomic DNA is prepared from the tail snips using, for example, a Qiagen Dneasy kit following the manufacturer's instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a IL-17RC gene or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

[346] To check for expression of a transgene in a live animal, a partial hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the zyphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. An atraumatic clamp is used to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with suture and wound clips, and the animal's cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of IL-17RC mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

[347] In addition to producing transgenic mice that over-express IL-17F, IL-17A, IL-17RA or IL-17RC, it is useful to engineer transgenic mice with either abnormally low or no expression of any of these genes. Such transgenic mice provide useful models for diseases associated with a lack of IL-17F, IL-17A, IL-17RA or IL-17RC. As discussed above, IL-17RC gene expression can be inhibited using anti-sense genes, ribozyme genes, or external guide sequence genes. For example, to produce transgenic mice that under-express the IL-17RC gene, such inhibitory sequences are targeted to IL-17RC mRNA. Methods for producing transgenic mice that have abnormally low expression of a particular gene are known to those in the art (see, for example, Wu et al., "Gene Underexpression in Cultured Cells and Animals by Antisense DNA and RNA Strategies," in Methods in Gene Biotechnology, pages 205-224 (CRC Press 1997)).

[348] An alternative approach to producing transgenic mice that have little or no IL-17RC gene expression is to generate mice having at least one normal IL-17RC allele replaced by a nonfunctional IL-17RC gene. One method of designing a nonfunctional IL-17RC gene is to insert another gene, such as a selectable marker gene, within a nucleic acid molecule that encodes IL-17RC. Standard methods for producing these so-called "knockout mice" are known to those skilled in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in Overexpression and Knockout of Cytokines in Transgenic Mice, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), and Wu et al., "New Strategies for Gene Knockout," in Methods in Gene Biotechnology, pages 339-365 (CRC Press 1997)).

[349] The invention is further illustrated by the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Expression of the IL-17RC Gene

[350] Northern analyses were performed using Human Multiple Tissue Blots (Clontech Laboratories, Inc., Palo Alto, CA). Two probes were generated from gel purified PCR products. The first probe was made using ZC21798 (5' CGG CGT GGT GGT CTT GCT CTT 3'; SEQ ID NO:8) and ZC21808 (5' TCC CGT CCC CCG CCC CAG GTC 3'; SEQ ID NO:31) as primers. The probe was a radioactively labeled using the Multiprime labeling kit from Amersham (Arlington Heights, IL) according to the manufacturer's protocol. The probe was purified using a NucTrap push column (Stratagene, La Jolla, CA). ExpressHyb (Clontech) solution was used for the prehybridization and hybridization solutions for the northern blots. Hybridization took place overnight at 65□C. Following hybridization, the blots were washed for 30 minutes each in solutions that contained 0.1% SDS and SSC as follows: twice in 2xSSC at room temperature, three times in 0.1x SSC at 50C°, once in 0.1x SSC at 55°C, and once in 0.1x SSC at 65°C. The results demonstrated the IL-17RC gene is strongly expressed in thyroid, adrenal gland, prostate, and liver tissues, and expressed to a lesser extent in heart, small intestine, stomach, and trachea tissues. In contrast, there is little or no expression in brain, placenta, lung, skeletal muscle, kidney, pancreas, spleen, thymus, testis, ovary, colon, peripheral blood leukocytes, spinal cord, lymph node, and bone marrow.

EXAMPLE 2

Distribution of mRNA in Cell Line Panels Using PCR

[351] Total RNA was purified from resting and stimulated cell lines grown in-house and purified using a Qiagen (Valencia, CA) RNeasy kit according to the manufacturer's instructions, or an acid-phenol purification protocol (Chomczynski and Sacchi, Analytical Biochemistry, 162:156-9, 1987). The quality of the RNA was assessed by running an aliquot on an Agilent Bioanalyzer. If the RNA was significantly degraded, it was not used for subsequent creation of first strand cDNA. Presence of contaminating genomic DNA was assessed by a PCR assay on an aliquot of the RNA with zc41011 (5'CTCTCCATCCTTATCTTTCATCAAC 3'; SEQ ID NO:32) and zc41012 (5'CTCTCTGCTGGCTAAACAAAACAC 3'; SEQ ID NO:33), primers that amplify a single site of intergenic genomic DNA. The PCR conditions for the contaminating genomic DNA assay were as follows: 2.5µl 10X buffer and 0.5µl Advantage 2 cDNA polymerase mix (BD Biosciences Clontech, Palo Alto, CA), 2ul 2.5mM dNTP mix (Applied Biosystems, Foster City, CA), 2.5µl 10X Rediload (Invitrogen, Carlsbad, CA), and 0.5µl 20uM zc41011 and zc41012, in a final volume of 25 ul. Cycling parameters were 94oC 20", 40 cycles of 94oC 20" 60oC 1'20" and one cycle of 72oC 7'. 10ul of each reaction was subjected to agarose gel electrophoresis and gels were examined for presence of a PCR product from contaminating genomic DNA. If contaminating genomic DNA was

observed, the total RNA was DNAsed using DNA-free reagents (Ambion, Inc, Austin, TX) according to the manufacturer's instructions, then retested as described above. Only RNAs which appeared to be free of contaminating genomic DNA were used for subsequent creation of first strand cDNA.

[352] 20μg total RNA from 82 human cell lines were each brought to 98μl with H2O, then split into two 49ul aliquots, each containing 10μg total RNA, and placed in two 96-well PCR plates. To each aliquot was added reagents for first strand cDNA synthesis (Invitrogen First Strand cDNA Synthesis System, Carlsbad, CA): 20μl 25mM MgCl2, 10ul 10X RT buffer, 10ul 0.1M DTT, 2μl oligo dT, 2ul RNAseOut. Then, to one aliquot from each cell line 2μl Superscript II Reverse Transcriptase was added, and to the corresponding cell line aliquot 2μl H2O was added to make a minus Reverse Transcriptase negative control. All samples were incubated as follows: 25oC 10', 42oC 50', 70oC 15'. Samples were arranged in deep well plates and diluted to 1.7ml with H2O. A Multipette (Saigan) robot was used to aliquot 16.5μl into each well of a 96-well PCR plate multiple times, generating numerous one-use PCR panels of the cell lines, which were then sealed and stored at –20oC. Each well in these panels represents first strand cDNA from approximately 100ng total RNA. The 82 cell lines are spread across two panels, array #118A and #118B. Quality of first strand cDNA on the panels was assessed by a multiplex PCR assay on one set of the panels using primers to two widely expressed, but only moderately abundant genes, CLTC (clathrin) and TFRC (transferrin receptor C). 0.5ul each of Clathrin primers zc42901 (5'CTCATATTGCTCAACTGTGTGAAAAG 3'; SEQ ID NO:34), zc42902(5'TAGAAGCCACCTGAACACAAATCTG3'; SEQ ID NO:35), and TFRC primers zc42599 (5'ATCTTGCGTTGTATGTTGAAAATCAATT3'; SEQ ID NO:36), zc42600 (5'TTCTCCACCAGGTAAACAAGTCTAC3'; SEQ ID NO:37), were mixed with 2.5μl 10X buffer and 0.5μl Advantage 2 cDNA polymerase mix (BD Biosciences Clontech, Palo Alto, CA), 2μl 2.5mM dNTP mix (Applied Biosystems,, Foster City, CA), 2.5μl 10X Rediload (Invitrogen, Carlsbad, CA), and added to each well of a panel of array#118A and array #118B. Cycling parameters were as follows: 94oC 20", 35 cycles of 94oC 20", 67oC 80", and one cycle of 72oC 7'. 10μl of each reaction was subjected to agarose gel electrophoresis and gels were scored for the presence of a robust PCR product for each gene specific to the +RT wells for each cell line.

[353] Expression of mRNA in the human first strand cDNA panels for IL-17RC was assayed by PCR with sense oligo ZC42756 (5'ctctccaggcccaagtcgtgctct3'; SEQ ID NO:38) and antisense oligo ZC42757 (5'ttgtcctgggggcctcgtgtctcc3'; SEQ ID NO:39) under these PCR conditions per sample: 2.5μl 10X buffer and 0.5μl advantage 2 cDNA polymerase mix (BD Biosciences Clontech, Palo Alto, CA), 2μl 2.5mM dNTP mix (Applied Biosystems,), 2.5ul 10X Rediload (Invitrogen, Carlsbad, CA), and 0.5μl 20uM each sense and antisense primer. Cycling conditions were 94oC 2', 35 cycles of 94oC 1', 66oC 30", 72oC 1.5', and one cycle of 72oC 7'. 10μl of each reaction

was subjected to agarose gel electrophoresis and gels were scored for positive or negative expression of IL-17RC.

[354] IL-17RC mRNA is widely expressed in many cell lines representing a broad spectrum of tissue and cell types. In particular, IL-17RC is consistently expressed in non-T cell peripheral blood cell lines, including monocytes, B-cells, and cells of the myeloid lineage. Also, IL-17RC mRNA is reliably expressed in cell lines derived from skin. Other cell lines that express IL-17RC are all 5 of the large intestine cell lines that were present on the array.

EXAMPLE 3

Distribution of mRNA in Mouse Cell Line Panels Using RT PCR

[355] Total RNA was purified from 60 resting and stimulated cell lines grown in-house and purified using a Qiagen (Valencia, CA) RNeasy kit according to the manufacturer's instructions, an acid-phenol purification protocol (Chomczynski and Sacchi, Analytical Biochemistry, 162:156-9, 1987), or a Trizol reagent protocol (Invitrogen, Carlsbad, CA).

[356] 5μg of total RNA from each cell line was arranged in a deep well 96-well plate, 125μl 3M NaOAc and 100μl Pellet Paint (Novagen, Madison, WI)) were added to each well, then the final volume was adjusted to 1.25ml with H20. A Multipette (Saigan) robot was used to aliquot 25μl of the RNA mixture followed by 75ul EtOH into each well of a 96-well PCR plate multiple times, generating numerous one-use RT PCR panels of the cell lines, which were then sealed and stored at –20oC. RT PCR screening was performed by first centrifuging a panel in a Qiagen (Valencia, CA) 96-well centrifuge for 10' at 6000 RPM. Supernatant was removed by inverting the plate onto absorbent paper. RNA pellets were washed with 100μl 70% EtOH, followed by a 5' centrifugation at 6000 RPM. Supernatant was again removed and plates allowed to air-dry until the remaining EtOH was evaporated. RNA pellets were resuspended in 15μl H20.

[357] Expression of IL-17RC mRNA in the mouse cell line RNA panels was assayed by RT PCR with zc38910 (5'acgaagcccaggtaccagaaagag3'; SEQ ID NO:40) and zc38679 (5'aaaagcgccgcagccaagagtagg3'; SEQ ID NO:41) under these RT PCR conditions per sample: SuperScript One-Step PCR with Platinum Taq kit, Invitrogen, Carlsbad, CA. Cycling conditions were:1 cycle of 48°C for 30 minutes, 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 1.5 minutes, followed by 1 cycle of 72°C for 7 minutes. 10μl of each reaction was subjected to agarose gel electrophoresis and gels were scored for positive or negative expression of IL-17RC.

[358] Murine IL-17RCmRNA is expressed in several mouse cell lines, notably in cell lines derived from bone marrow, including osteoblast, adipocyte, and preadipocyte cell lines. Also, mouse IL-17RC is mRNA is represented in several samples from the endocrine system, such as pancreas stromal cell lines, pancreas islet cell lines, and hypothalamus, salivary gland, and testis cell lines.

EXAMPLE 4

Refolding and Purification pIL-17F Produced in *E.coli*

A) Inclusion body isolation and extraction of pIL-17F

[359] Following induction of protein expression in either batch ferment or shaker flask culture, the E.coli broth is centrifuged in 1 liter bottles @ 3000 RPM in a Sorvall swinging bucket rotor. Washing of the cell paste to remove any broth contaminants is performed with 50 mM Tris pH 8.0 containing 200 mM NaCl and 5 mM EDTA until the supernate is clear.

[360] The cell pellets are then suspended in ice-cold lysis buffer (50 mM Tris pH 8.0; 5 mM EDTA; 200 mM NaCl, 10% sucrose (w/v); 5mM DTT; 5 mM Benzamidine;) to 10-20 Optical Density units at 600 nm. This slurry is then subjected to 3 passes at 8500-9000 psi in a chilled APV 2000 Lab Homogenizer producing a disrupted cell lysate. The insoluble fraction (inclusion bodies) is recovered by centrifugation of the cell lysate at 20,000 X G for 1 hour at 4°C.

[361] The inclusion body pellet resulting from the 20,000 X G spin is weighed and then re-suspended in wash buffer (50 mM Tris pH 8 containing 200 mM NaCl, 5 mM EDTA, 5mM DTT, 5mM Benzamidine) at 10 ml wash buffer per gram inclusion bodies. Complete dispersion is achieved by homogenizing with an OMNI international rotor stator generator. This suspension is centrifuged at 20,000 X G for 30 minutes at 4°C. The wash cycle is repeated 3-5 times until the supernatant is clear.

[362] The final washed pellet is solubilized in 7M Guanidine HCl in 40 mM Tris buffer at pH 8 containing 0.1M Sodium Sulfite and 0.02 M Sodium Tetrathionate. The extraction and sulfitolysis reaction is allowed to proceed with gentle stirring at 4°C overnight. The resulting pinkish colored solution is centrifuged at 35,000 X g for 1 hour at 4°C and the clarified supernate, containing the soluble pIL-17F, is 0.45 um filtered.

B) pIL-17F refolding procedure

[363] The solubilized, sulfitolyzed pIL-17F is refolded by drop wise dilution into ice cold refolding buffer containing 55 mM MES, 10.56 mM NaCl, 0.44 mM KCl, 0.055% PEG (3400 K), 1.1 mM EDTA, 20% Glycerol, 0.5M Guanidine HCl, 0.75 M Arginine and the Glutathione redox pair at a 1:1 ratio (1mM GSH : 1mM GSSG). The pH of the refolding buffer is adjusted to 6.5 with HCl and the pIL-17F is added to a final concentration of 100 ug/ml. Once diluted, the mixture is allowed to stir slowly in the cold room for 72 hours.

C) Product recovery & purification

[364] The refolded pIL-17F is concentrated 10X vs. a 10kDa cutoff membrane on a lab scale TFF system. Next it is filtered using a 0.45 micron membrane and the pH is adjusted to 5.1 with the addition of Acetic acid. The pH-adjusted material is captured by cation exchange chromatography on a Pharmacia SP Fast Flow column equilibrated in 50 mM Acetate buffer, pH 5.1.

The pIL-17F is loaded by inline proportioning at 1:5 with equilibration buffer at a flow rate of 190 cm/hr. This dilution lowers the ionic strength enabling efficient binding of the target to the matrix. After sample loading is complete, the column is washed to baseline absorbance with equilibration buffer. The column is washed with 0.4 M NaCl in 50 mM Acetate buffer at pH 5.1 and then the bound protein is eluted with a 5 CV gradient from 0.4 M to 1.5 M NaCl in 50 mM Acetate buffer at pH 5.1. The protein elutes at ~ 1M NaCl and is approximately 85% dimeric by SDS PAGE analysis of eluate fractions. The fractions containing pIL-17F are pooled and concentrated against a 10 kDa cutoff ultrafiltration membrane using an Amicon stirred cell in preparation for the final purification and buffer exchange by size exclusion chromatography.

D) Size exclusion buffer exchange and formulation

[365] The concentrated cation pool (at a volume of 3-4% of CV) is injected at a flow rate of 30 cm/hr onto a Pharmacia Superdex 75 size exclusion column equilibrated in 50 mM Sodium Phosphate buffer containing 109 mM NaCl, pH 7.2. The symmetric eluate peak containing the product is diluted to a concentration of 1 mg/ml in 50 mM Sodium Phosphate buffer containing 109 mM NaCl, pH 7.2. Finally the pIL-17F is 0.2 micron sterile filtered, aliquoted and stored at –80°C. The final process yield is 20%.

EXAMPLE 5

Construction of Mammalian Soluble IL-17RC Expression Construct

[366] An expression construct containing human IL-17RC [L21-K451]-mFc1 (mouse BALB/c μ2a Fc) is constructed via overlap PCR and homologous recombination using a DNA fragment (SEQ ID NO:42) encoding a IL-17RC polypeptide (SEQ ID NO:43), a DNA fragment encoding mFc1 (SEQ ID NO:44), and the expression vector pZMP20. The fragments are generated by PCR amplification.

[367] The PCR fragment encoding IL-17RC [L21-K451] contains a 5' overlap with the pZMP20 vector sequence in the optimized tissue plasminogen activator pre-pro secretion leader sequence coding region, the IL-17RC extracellular domain coding [L21-K451], and a 3' overlap with the mFc1 coding region. The PCR amplification reaction uses the 5' oligonucleotide [GTTTCGCTCAGCCAGGAAATCCATGCCGAGTTGAGACGCTTCCGTAGACTGGAGAGGCT TGTGGGGCCT; SEQ ID NO:46], the 3' oligonucleotide [TGTGGGCCCTCTGGGCTCCTTGTGGATGTATTTGTC; SEQ ID NO:47], and a previously generated DNA clone of IL-17RC as the template.

[368] The PCR fragment encoding mFc1 contains a 5' overlap with the IL-17RC sequence, the mFc1 coding region, and a 3' overlap with the pZMP20 vector in the poliovirus internal ribosome entry site region. The PCR amplification reaction uses the 5 oligonucleotide [GACAAATACATCCACAAGGAGCCCAGAGGGCCCACA; SEQ ID NO:48], the 3'

oligonucleotide [CAACCCCAGAGCTGTTTTAAGGCGCGCCTCTAGATTATTTACCCGGAGTCCGGGA; SEQ ID NO:49], and a previously generated DNA clone of mFc1 as the template.

[369] The PCR amplification reaction conditions are as follows: 1 cycle, 94 °C, 5 minutes; 35 cycles, 94 °C, 1 minute, followed by 55 °C, 2 minutes, followed by 72 °C, 3 minutes; 1 cycle, 72 °C, 10 minutes. The PCR reaction mixtures are run on a 1% agarose gel and the DNA fragments corresponding to the expected sizes are extracted from the gel using a QIAquick™ Gel Extraction Kit (Qiagen, Cat. No. 28704).

[370] The two PCR fragments are joined by overlap PCR. Approximately 1 µl each of the two gel extracted fragments are combined in a PCR amplification reaction using the 5' oligonucleotide [GTTTCGCTCAGCCAGGAAATCCATGCCGAGTTGAGACGCTTCCGTAGACTGGAGAGGCT TGTGGGGCCT; SEQ ID NO: 46] and the 3' oligonucleotide [CAACCCCAGAGCTGTTTTAAGGCGCGCCTCTAGATTATTTACCCGGAGTCCGGGA; SEQ ID NO:49]. PCR conditions used are as follows: 1 cycle, 94 °C, 5 minutes; 35 cycles, 94 °C, 1 minute, followed by 55 °C, 2 minutes, followed by 72 °C, 3 minutes; 1 cycle, 72 °C, 10 minutes. The PCR reaction mixture is run on a 1% agarose gel and the DNA fragment corresponding to the size of the insert is extracted from the gel using a QIAquick™ Gel Extraction Kit (Qiagen, Cat. No. 28704).

[371] Plasmid pZMP20 is a mammalian expression vector containing an expression cassette having the MPSV promoter, a BglII site for linearization prior to yeast recombination, an otPA signal peptide sequence, an internal ribosome entry element from poliovirus, the extracellular domain of CD8 truncated at the C-terminal end of the transmembrane domain; an E. coli origin of replication; a mammalian selectable marker expression unit comprising an SV40 promoter, enhancer and origin of replication, a DHFR gene, and the SV40 terminator; and URA3 and CEN-ARS sequences required for selection and replication in S. cerevisiae.

[372] The plasmid pZMP20 is digested with BglII prior to recombination in yeast with the gel extracted IL-17RC[L21-K451]-mFc1 PCR fragment. 100µl of competent yeast (S. cerevisiae) cells are combined with 10µl of the IL-17RC[L21-K451]-mFc1 insert DNA and 100 ng of BglII digested pZMP20 vector, and the mix is transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture is electropulsed using power supply (BioRad Laboratories, Hercules, CA) settings of 0.75 kV (5 kV/cm), ∞ ohms, and 25 µF. Six hundred µl of 1.2 M sorbitol is added to the cuvette, and the yeast is plated in 100 µl and 300 µl aliquots onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate are resuspended in 1 ml H2O and spun briefly to pellet the yeast cells. The cell pellet is resuspended in 0.5 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The five hundred µl of the lysis mixture is added to an Eppendorf tube containing 250 µl acid-washed glass beads and 300 µl

phenol-chloroform, is vortexed for 3 minutes, and spun for 5 minutes in an Eppendorf centrifuge at maximum speed. Three hundred µl of the aqueous phase is transferred to a fresh tube, and the DNA is precipitated with 600 µl ethanol, followed by centrifugation for 30 minutes at maximum speed. The tube is decanted and the pellet is washed with 1 mL of 70% ethanol. The tube is decanted and the DNA pellet is resuspended in 30 µl 10 mM Tris, pH 8.0, 1 mM EDTA.

[373] Transformation of electrocompetent E. coli host cells (DH12S) is done using 5 µl of the yeast DNA preparation and 50 µl of E. coli cells. The cells are electropulsed at 2.0 kV, 25 µF, and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose) is added and then the cells are plated in 50 µl and 200 µl aliquots on two LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

[374] The inserts of three DNA clones for the construct is subjected to sequence analysis and one clone containing the correct sequence is selected. Large scale plasmid DNA is isolated using a commercially available kit (QIAGEN Plasmid Mega Kit, Qiagen, Valencia, CA) according to manufacturer's instructions.

EXAMPLE 6

Construction of Mammalian Soluble IL-17RC Expression Constructs that Express IL-17RC-CEE, IL-17RC-CHIS, and IL-17RC-CFLAG

[375] An expression construct containing human IL-17RC [L21-K451] with a C-terminal tag, either Glu-Glu (CEE), six His (CHIS), or FLAG (CFLAG), is constructed via PCR and homologous recombination using a DNA fragment encoding IL-17RC [L21-K451] (SEQ ID NO:42) and the expression vector pZMP20.

[376] The PCR fragment encoding IL-17RCCEE contains a 5' overlap with the pZMP20 vector sequence in the optimized tissue plasminogen activator pre-pro secretion leader sequence coding region, the IL-17RC extracellular domain coding [L21-K451], the sequence of the Glu-Glu tag (Glu Glu Tyr Met Pro Met Glu; SEQ ID NO:53), and a 3' overlap with the pZMP20 vector in the poliovirus internal ribosome entry site region. The PCR amplification reaction uses the 5' oligonucleotide [GTTTCGCTCAGCCAGGAAATCCATGCCGAGTTGAGACGCTTCCGTAGACTGGAGAGGCT TGTGGGGCCT; SEQ ID NO:46], the 3' oligonucleotide [CAACCCCAGAGCTGTTTTAAGGCGCGCCTCTAGATTATTCCATGGGCATGTATTCTTCCT TGTGGATGTATTTGTC; SEQ ID NO:50], and a previously generated DNA clone of IL-17RC as the template.

[377] The PCR amplification reaction condition is as follows: 1 cycle, 94 °C, 5 minutes; 35 cycles, 94 °C, 1 minute, followed by 55 °C, 2 minutes, followed by 72 °C, 3 minutes; 1 cycle, 72 °C,

10 minutes. The PCR reaction mixture is run on a 1% agarose gel and the DNA fragment corresponding to the expected size is extracted from the gel using a QIAquick™ Gel Extraction Kit (Qiagen, Cat. No. 28704).

[378] The plasmid pZMP20 is digested with BglII prior to recombination in yeast with the gel extracted IL-17RCCEE PCR fragment. One hundred µl of competent yeast (S. cerevisiae) cells are combined with 10 µl of the IL-17RCCEE insert DNA and 100 ng of BglII digested pZMP20 vector, and the mix is transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture is electropulsed using power supply (BioRad Laboratories, Hercules, CA) settings of 0.75 kV (5 kV/cm), ∞ ohms, and 25 µF. Six hundred µl of 1.2 M sorbitol is added to the cuvette, and the yeast is plated in 100 µl and 300 µl aliquots onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate are resuspended in 1 ml H2O and spun briefly to pellet the yeast cells. The cell pellet is resuspended in 0.5 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The five hundred µl of the lysis mixture is added to an Eppendorf tube containing 250 µl acid-washed glass beads and 300 µl phenol-chloroform, is vortexed for 3 minutes, and spun for 5 minutes in an Eppendorf centrifuge at maximum speed. Three hundred µl of the aqueous phase is transferred to a fresh tube, and the DNA is precipitated with 600 µl ethanol, followed by centrifugation for 30 minutes at maximum speed. The tube is decanted and the pellet is washed with 1 mL of 70% ethanol. The tube is decanted and the DNA pellet is resuspended in 30 µl 10 mM Tris, pH 8.0, 1 mM EDTA.

[379] Transformation of electrocompetent E. coli host cells (DH12S) is done using 5 µl of the yeast DNA preparation and 50 µl of E. coli cells. The cells are electropulsed at 2.0 kV, 25 µF, and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose) is added and then the cells are plated in 50 µl and 200 µl aliquots on two LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

[380] The inserts of three DNA clones for the construct is subjected to sequence analysis and one clone containing the correct sequence is selected. Large scale plasmid DNA is isolated using a commercially available kit (QIAGEN Plasmid Mega Kit, Qiagen, Valencia, CA) according to manufacturer's instructions.

[381] The same process is used to prepare the IL-17RC with a C-terminal his tag, composed of Gly Ser Gly Gly His His His His His His (IL-17RCCHIS; SEQ ID NO:51) or the C-terminal FLAG tag , composed of Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys (IL-17RCCFLAG; SEQ ID NO:52). To prepare these constructs, instead of the 3' oligonucleotide of SEQ ID NO:50; the 3' oligonucleotide
[CAACCCCAGAGCTGTTTTAAGGCGCGCCTCTAGATTAGTGATGGTGATGGTGATGTCCA CCAGATCCCTTGTGGATGTATTTGTC; SEQ ID NO:54] is used to generate IL-17RCCHIS or the

3' oligonucleotide [CAACCCCAGAGCTGTTTTAAGGCGCGCCTCTAGATTACTTATCATCATCATCCTTATAAT CGGATCCCTTGTGGATGTATTTGTC; SEQ ID NO:55] is used to generate IL-17RCCFLAG.

EXAMPLE 7

Transfection and Expression of Soluble IL-17RC Receptor Expression Constructs that Express the IL-17RC-mFc1 Fusion Protein, and the IL-17RC-CEE, IL-17RC-CHIS, and IL-17RC-CFLAG C-Terminal Tagged Proteins

[382] Three sets of 200 μg of each of the soluble IL-17RC fusion or tagged expression constructs are separately digested with 200 units of PvuI at 37°C for three hours, precipitated with isopropyl alcohol, and centrifuged in a 1.5 mL microfuge tube. The supernatant is decanted off the pellet, and the pellet is washed with 1 mL of 70% ethanol and allowed to incubate for 5 minutes at room temperature. The tube is spun in a microfuge for 10 minutes at 14,000 RPM and the supernatant is decanted off the pellet. The pellet is then resuspended in 750 μl of CHO cell tissue culture medium in a sterile environment, allowed to incubate at 60o C for 30 minutes, and is allowed to cool to room temperature. Approximately 5 x 106 CHO cells are pelleted in each of three tubes and are resuspended using the DNA-medium solution. The DNA/cell mixtures are placed in a 0.4 cm gap cuvette and electroporated using the following parameters; 950 μF, high capacitance, at 300 V. The contents of the cuvettes are then removed, pooled, and diluted to 25 mLs with CHO cell tissue culture medium and placed in a 125 mL shake flask. The flask is placed in an incubator on a shaker at 37 °C, 6% CO2 with shaking at 120 RPM.

[383] The CHO cells are subjected to nutrient selection followed by step amplification to 200 nM methotrexate (MTX), and then to 1 μM MTX. Fusion or tagged protein expression is confirmed by Western blot, and the CHO cell pool is scaled-up for harvests for protein purification.

EXAMPLE 8

Expression of Soluble IL-17RC

[384] An expression plasmid containing IL-17RC-Tbx-C(Fc9) (SEQ ID NO:64) was constructed via homologous recombination using a DNA fragment of IL-17RC _Tbx and the expression vector pZMP40. The fragment was generated by PCR amplification using primers zc44531 and zc44545.

[385] The PCR fragment IL-17RC_Tbx contains a partial IL-17RC extracellular domain coding region, which was made using a previously generated clone of IL-17RC as the template. The fragment includes a 5' overlap with the pZMP40 vector sequence in the otPA coding region, the IL-17RC segment (amino acid residue 21 to 451 of SEQ ID NO:2), a linker sequence, a thrombin cleavage site, and a 3' overlap with the pZMP40 vector in the Fc9 coding region. PCR conditions

used were as follows: 1 cycle, 94°C, 5 minutes; 35 cycles, 94°C, 1 minute, followed by 55°C, 2 minutes, followed by 72°C, 3 minutes; 1 cycle, 72°C, 10 minutes.

[386] The PCR reaction mixtures were run on a 1% agarose gel and a band corresponding to the sizes of the inserts were gel-extracted using a QIAquick™ Gel Extraction Kit (Qiagen, Cat. No. 28704).

[387] Plasmid pZMP40 is a mammalian expression vector containing an expression cassette having the MPSV promoter, multiple restriction sites for insertion of coding sequences, an otPA signal peptide sequence, and the sequence for Fc9; an internal ribosome entry site (IRES) element from poliovirus, and the extracellular domain of CD8 truncated at the C-terminal end of the transmembrane domain; an E. coli origin of replication; a mammalian selectable marker expression unit comprising an SV40 promoter, enhancer and origin of replication, a DHFR gene, and the SV40 terminator; and URA3 and CEN-ARS sequences required for selection and replication in S. cerevisiae. It was constructed from pZMP21 (Patent Pub. No. US 2003/0232414 A1; deposited at the American Type Culture Collection and designated as ATCC# PTA-5266).

[388] The plasmid pZMP40 was cut with BglII prior to recombination in yeast with the PCR fragment. One hundred microliters of competent yeast (S. cerevisiae) cells were independently combined with 10 µl of the insert DNA (SEQ ID NO:66) and 100ng of cut pZMP40 vector, and the mix was transferred to a 0.2-cm electroporation cuvette. The yeast/DNA mixture was electropulsed using power supply (BioRad Laboratories, Hercules, CA) settings of 0.75 kV (5 kV/cm), ∞ ohms, and 25 µF. Six hundred µl of 1.2 M sorbitol was added to the cuvette, and the yeast was plated in a 100-µl and 300µl aliquot onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H2O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 0.5 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The five hundred microliters of the lysis mixture was added to an Eppendorf tube containing 250 µl acid-washed glass beads and 300 µl phenol-chloroform, was vortexed for 3 minutes, and spun for 5 minutes in an Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA was precipitated with 600 µl ethanol (EtOH), followed by centrifugation for 30 minutes at maximum speed. The tube was decanted and the pellet was washed with 1 mL of 70% ethanol. The tube was decanted and the DNA pellet was resuspended in 30 µl TE.

[389] Transformation of electrocompetent E. coli host cells (DH12S) was done using 5 µl of the yeast DNA prep and 50 µl of cells. The cells were electropulsed at 2.0 kV, 25 µF, and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose) was added and then the cells were plated in a 50 µl and a 200 µl aliquot on two LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

[390] The inserts of three clones for the construct was subjected to sequence analysis and one clone for each construct, containing the correct sequence, was selected. Larger scale plasmid DNA was isolated using a commercially available kit (QIAGEN Plasmid Mega Kit, Qiagen, Valencia, CA) according to manufacturer's instructions.

[391] Three sets of 200μg of the IL-17RC[L21-K451]_Tbx_C(Fc9) construct were then each digested with 200 units of Pvu I at 37°C for three hours and then were precipitated with IPA and spun down in a 1.5 mL microfuge tube. The supernatant was decanted off the pellet, and the pellet was washed with 1 mL of 70% ethanol and allowed to incubate for 5 minutes at room temperature. The tube was spun in a microfuge for 10 minutes at 14,000 RPM and the supernatant was decanted off the pellet. The pellet was then resuspended in 750 μl of PF-CHO media in a sterile environment, allowed to incubate at 60oC for 30 minutes, and was allowed to cool to room temperature. 5E6 APFDXB11 cells were spun down in each of three tubes and were resuspended using the DNA-media solution. The DNA/cell mixtures were placed in a 0.4 cm gap cuvette and electroporated using the following parameters: 950 μF, high capacitance, and 300 V. The contents of the cuvettes were then removed, pooled, and diluted to 25 mLs with PF-CHO media and placed in a 125 mL shake flask. The flask was placed in an incubator on a shaker at 37°C, 6% CO2, and shaking at 120 RPM.

[392] The cell line was subjected to nutrient selection followed by step amplification to 200nM methotrexate (MTX), and then to 1μM MTX. Expression was confirmed by western blot, and the cell line was scaled-up and protein purification followed.

EXAMPLE 9

Purification of Soluble IL-17RC from CHO Cells

[393] Conditioned media from CHO cells expressing IL-17RC-TbX-Fc9 (SEQ ID NO:64) was concentrated approximately 10-fold with a Pellicon-II tangential flow system against two Biomax 0.1 m2 30kD molecular weight cutoff membrane cassettes (Millipore, Bedford, MA). The concentrated media was pH adjusted to 5.5 with glacial acetic acid, 0.2 □m sterile filtered then loaded onto a Protein G sepharose fast flow resin (Pharmacia, Piscataway, NJ) via batch chromatography overnight at 4C. Prior to loading the pH adjusted conditioned media, the Protein G resin was pre-equilibrated with, 5 column volumes (approximately 150ml) of 25mM sodium acetate, 150mM NaCl, pH5.5. The ratio of filtered, pH adjusted conditioned media to resin was 33:1 (v/v).

[394] The batched chromatography process was performed at ambient room temperature (approximately 21C). The batched, pH adjusted, 0.22 μm filtered, conditioned media was poured into an empty 5.5 x 20.5 cm glass column (BioRad, Hercules, CA) and packed via gravity. The column was washed with 10 column volumes (approximately 300ml) of 25mM sodium acetate, 150mM NaCl, pH5.5. Bound protein was then pH eluted with 100mM glycine, pH 2.7. 9.0ml fractions were collected and immediately neutralized with 1.0 ml 2.0M Tris, pH 8.0. The collected fractions were

analyzed via SDS-PAGE Coomassie staining. Fractions containing IL-17RC-Tbx-Fc9 were pooled and concentrated approximately 6-fold using a 5kD molecular weight cutoff Biomax membrane spin concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions.

[395] The pooled, concentrated fractions were then dialyzed ,at 4C, extensively against 1X phosphate buffered saline, pH 7.3 (Sigma, St. Louis, MO) using a 7kD molecular weight cutoff membrane Slide-A-Lyzer (Pierce, Rockford, IL). IL-17RC-TbX-Fc9 as formulated in 1x phosphate buffered saline, pH 7.3 was 0.22 μm sterile filtered prior to aliquoting and storage at -80C.

EXAMPLE 10

Binding of IL-17A and IL-17F to Human IL-17RC

A) Binding of biotinylated cytokines to transfected cells

[396] Baby Hamster Kidney (BHK) cells that had been transfected with expression vectors encoding human IL-17 receptor (SEQ ID NO:21), human IL-17RC (SEQ ID NO:2), or both of these receptors are assessed for their ability to bind biotinylated human IL-17A and human IL-17F. Cells are harvested with versene, counted and diluted to 107 cells per ml in staining media (SM), which is HBSS plus 1 mg/ml bovine serum albumin (BSA), 10 mM Hepes, and 0.1% sodium azide (w/v). Biotinylated human IL-17A (SEQ ID NO:14) and human IL-17F (SEQ ID NO:16) are incubated with the cells on ice for 30 minutes at various concentrations. After 30 minutes, excess cytokine is washed away with SM and the cells are incubated with a 1:100 dilution of streptavidin conjugated to phycoerythrin (SA-PE) for 30 minutes on ice. Excess SA-PE is washed away and cells are analyzed by flow cytometry. The amount of cytokine binding was quantitated from the mean fluorescence intensity of the cytokine staining. From this analysis, we find that human IL-17A binds both the human IL-17R and IL-17RC to a similar extent. Also, human IL-17F binds IL-17RC to a similar level, but binds IL-17R detectably, but to a much lower level than was seen with IL-17A.

B) Binding of biotinylated cytokines to human peripheral blood mononuclear cells

[397] Human peripheral blood mononuclear cells (PBMC) were prepared from whole blood by ficoll density gradient centrifugation. PBMC at 107 cells per ml were simultaneously incubated with biotinylated IL-17A or IL-17F at 1 μg/ml and fluorochrome conjugated antibodies to specific cell surface proteins that were designed to distinguish various white blood cell lineages lineages. These markers include CD4, CD8, CD19, CD11b, CD56 and CD16. Excess antibody and cytokine are washed away, and specific cytokine binding is detected by incubating with SA-PE as described above. Samples were analyzed by flow cytometry and from this analysis, we find that human IL-17A binds to virtually all PBMC populations examined, but that human IL-17F does not detectably bind to any population.

C) Inhibition of specific binding of biotinlyated human IL-17A and IL-17F with unlabeled cytokine

[398] Binding studies are performed as discussed above, but excess unlabeled human IL-17A and IL-17F are included in the binding reaction. In studies with BHK cells, the amount of unlabeled cytokine was varied over a range of concentrations and we find that addition of unlabeled IL-17A competed for binding of both IL-17A and IL-17F to both IL-17RC and IL-17R. However, unlabeled IL-17F competed for binding of both IL-17A and IL-17F to IL-17RC, but it did not compete effectively for binding to IL-17R. This indicates that both IL-17A and IL-17F specifically bind to IL-17RC, and that they bind at a site that is either identical or overlaps significantly since they cross-compete for binding. Also, IL-17A competes for the relatively weak binding of IL-17F for IL-17R, indicating these two cytokines also bind to a similar region in the IL-17R, but IL-17F binds IL-17R with much reduced affinity relative to IL-17RC.

D) Inhibition of specific binding of biotinylated human IL-17A and IL-17F with soluble IL-17RC and IL-17R

[399] Binding studies are performed as discussed above, except that a soluble form of IL-17RC or IL-17R are included in the binding reactions. These soluble receptors are fusion proteins derived from the extracellular domain of each receptor fused to the human IgG1 constant (Fc) region. We find that soluble IL-17RC inhibits binding of both human IL-17A and IL-17F to both IL-17R and IL-17RC transfected BHK cells. However, soluble IL-17R inhibits binding of IL-17A to either receptor, but does not effectively block binding of IL-17F to IL-17RC, consistent with the poor binding of IL-17F for the IL-17R.

EXAMPLE 11

IL-17A and IL-17F Bind to IL-17RC

A) Binding Inhibition with Cold Ligand

[400] BHK cells transfected with hIL-17RC (SEQ ID NO:2) and IL-17R (SEQ ID NO:21) were plated at 40,000 cells/well in a 24-well dish (Costar 3527)two days prior to assay. IL-17A (SEQ ID NO:14) and IL-17F(SEQ ID NO:16) that had been radiolabeled by the iodobead method were added independently to wells in triplicate at 10ng/ml with a total of 250ul/well in binding buffer (RPMI 1640 media (JRH 51502-500M) with 10mg/ml bovine serum albumin(Gibco15260-037)). Cold competitors were added in 100 fold molar excess. Competitors tested included IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F and IL-21. Wells were incubated on ice for 1-hour followed by two washes with PBS (Invitrogen 20012-027) and one wash with a high salt solution (1.5M NaCL, 50mM HEPES pH 7.4). Wells were extracted with 500ul of 0.8M NaOH for 30min. at room temperature and counts per minute were measured in a gamma counter (Packard Cobra II A5005).

[401] The results indicated that 100x molar cold IL-17A and IL-17F were able to reduce binding of 125I IL-17A to BHK hIL-17RC by approximately 7 fold while IL-17B,C,D,E and IL-21 had no effect on binding. 100x molar cold IL-17A reduced the binding of 125I IL-17A to BHK IL-

17R by approximately 4 fold while IL-17B,C,D,E,F and IL-21 had no effect on binding. 100x molar cold IL-17A and IL-17F reduced the binding of 125IL-17F to BHK hIL-17RC by approximately 4 fold and 5 fold, respectively, while IL-17B,C,D,E and IL-21 had no effect on binding.

B) Binding Inhibition with Soluble Receptor:

[402] Binding to hzytor14 (SEQ ID NO:2) and IL-17R (SEQ ID NO:21) transfected BHK cells was performed as in one, but 100 fold molar excess soluble hIL-17RCx1/Fc9 (Example 8) and soluble IL-17R/Fc (obtained from R&D; Ref. 177-IR) were used in place of cold ligand in the competition. Cells were washed, extracted and counted as in part one.

[403] Soluble hIL-17RC/Fc inhibited binding of 125IL-17F to BHK hIL-17RC with an IC50 of 10X molar excess average from three experiments. Soluble hIL-17RC/Fc inhibition of 125IIL-17A on the same cell line gave an average IC50 of 20X molar excess and soluble IL-17R/Fc inhibition of 125I IL-17A gave an average IC50 of 20X molar excess.

C) Binding Saturation

[404] Transfected BHK cells were plated into 24-well dishes as in one. Radiolabeled IL-17A and IL-17F were added starting at a concentration of 4nM in eight 1:3 dilutions (to a concentration of 1.83 pM) in triplicate with a total of 250µl/well in binding buffer. Separately, 100 fold molar excess of cold ligand was added at each dilution point. Cells were washed, extracted and counted as in one. Specific counts per minute were plotted against concentration of radiolabeled ligand added by subtracting the 100 fold excess counts from the the uncompeted counts at each dilution point. These normalized data were plotted to generate saturation binding curves for each combination of radiolabeled ligand and transfected BHK cells. Table 7 shows the affinity values calculated from all three experiments.

Table 7

125I IL-17A + BHK hIL-17RC	125I IL-17A + BHK IL-17R
1. 180pM	1. 2.5 +/- 0.2nM
2. 200pM	2. 4.5 +/- 0.3nM
3. 370pM	3. 5.9 +/- 0.1nM
125I IL-17F + BHK hIL-17RC	125I IL-17F + BHK IL-17R
1. 50pM	1. Very low affinity
2. 60pM	2. Very low affinity
3. 80pM	3. Very low affinity

[405] One-site binding curve fits agreed most closely with IL-17A & IL-17F binding to IL-17R. Two-site binding curve fits agreed most closely with IL-17A and IL-17F binding to hIL-17RC.

The high affinity binding site is the value shown above. The low affinity binding site had very low affinity and varied widely between the three experiments.

EXAMPLE 12

Murine Nih3t3 Cells Respond to Human IL-17A and IL-17F

A) Cell plating and kz142 adenovirus reporter infection.

[406] Nih3t3 cells, derived from mouse fibroblasts (described in ATCC) Nih3t3 were plated at 5000 cells/well in solid white, cell culture coated 96 well plates, (Cat. #3917. Costar) using DMEM/10% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02. On this second day, the plating media was removed and Kz142 adenovirus particles at a multiplicity of infection of 5000 particles/cell were prepared in DMEM/1% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02.

B) Luciferase assay measuring IL-17A and F activation of kz142 adenovirus reporter infected nih3t3 cells.

[407] Following the overnight incubation with the adenovirus particle reporter, human IL-17A and IL-17F Ligand treatments were prepared in serum free media ()amended to .28% BSA. The adenovirus particles and media were removed and the appropriate ligand doses were given in triplicates. Incubation at 37°C and 5% C02 was continued for 4 hours, after which the media was removed, cells lysed for 15 minutes and mean fluorescence intensity (MFI) measured using the luciferase assay system and reagents. (Cat.#e1531 Promega. Madison, WI.) and a Microplate luminometer. Activity was detected at concentrations ranging from .1-1000ng/ml human IL-17A and IL-17F, generating EC50 values of about 50ng/ml for both ligands. These data suggest that nih3t3 cells carry receptors to these ligands and that IL-17A and IL-17F activate the NfKb/Ap-1 transcription factor.

EXAMPLE 13

Murine Nih3t3 Cells Express Both IL-17RA and IL-17RC

RTPCR analysis of nih3t3 RNA demonstrated that these cells are positive for both IL-17 RA and IL-17RC, consistent with their nfkb/ap1 response to human IL-17A and IL-17F mediation being mediated through one or both of these receptors.

RTPCR DETAILS:

A) Murine IL-17RC PCR

[408] First strand cDNA was prepared from total RNA isolated from nih3t3 cells using standard methods. PCR was applied using hot star polymerase and the manufacturer's recommendations (Qiagen, Valencia, CA) using sense primer, zc38910, 5' ACGAAGCCCAGGTACCAGAAAGAG 3' (SEQ ID NO:56) and antisense primer, zc 38679, 5'

AAAAGCGCCGCAGCCAAGAGTAGG 3' (SEQ ID NO:57) and 35 cycles of amplification. Agarose gel electrophoresis revealed a single, robust amplicon of the expected, 850 bp size.

B) Murine IL-17RA PCR

[409] First strand cDNA was prepared from total RNA isolated from nih3t3 cells using standard methods. PCR was applied using hot star polymerase and the manufacturer's recommendations (Qiagen, Valencia, CA) using sense primer, zc38520, 5' CGTAAGCGGTGGCGGTTTTC 3'(SEQ ID NO:58) and antisense primer, zc 38521, 5' TGGGCAGGGCACAGTCACAG 3' (SEQ ID NO:59) and 35 cycles of amplification. Agarose gel electrophoresis revealed a single, robust amplicon of the expected, 498 bp size.

EXAMPLE 14

Creation of a Stable Nih3t3 Assay Clone Expressing the ap1/nfkb Transcription Factor

[410] The murine nih3t3 cell line described above was stably transfected with the kz142 ap1/nfkb reporter construct, containing a neomycin-selectible marker. The Neo resistant transfection pool was plated at clonal density. Clones were isolated using cloning rings and screened by luciferase assay using the human IL-17A ligand as an inducer. Clones with the highest mean fluorescence intensity (MFI) (via ap1/NfkB luciferase) and the lowest background were selected. A stable transfectant cell line was selected and called nih3t3/kz142.8.

EXAMPLE 15

Inhibition of Activation by Human IL-17A and IL-17F in Murine Nih3t3 Cells Using Soluble IL-17RC and IL-17RA/FC Chimeras

[411] Soluble forms of IL-17RC or IL-17RA were used as antagonists of human IL-17A and IL-17F activation of ap1/nfkb elements in a luciferase assay. These soluble receptors are fusion proteins derived from the extracellular domain of each receptor fused to the human IgG1 constant (Fc) region. The soluble human IL-17R FC fusion protein was purchased. (recombinant human IL-17R/FC chimera, catalog number 177-IR-100, R&D Systems, Inc., Minneapolis, Mn.) The soluble human IL-17RC FC chimera (IL-17RCsR/FC9) was constructed as described above. We find that an excess IL-17RCsR/FC9 and human IL17RsR/FC chimera inhibit EC50 levels of both human IL-17A and IL-17F mediation of ap1/nfkb activation of the murine nih3t3/kz142.8 assay cell line.

[412] The IL-17RCsR/FC9 protein showed the greatest potency in antagonizing IL-17F activation and IL17RsR/FC chimera showed the greatest potency in antagonizing IL-17A activation.

EXAMPLE 16

IL-17F mRNA is Upregulated in a Murine Model of Asthma

[413] IL-17F mRNA levels were measured in a sensitization and airway challenge model in mice. Groups of mice, 8 to 10 wks of age, were sensitized by intraperitoneal injection of 10 ug of recombinant Dermatophagoides pteronyssinus allergen 1 (DerP1) (Indoor biotechnologies, Cardiff, UK) in 50 % Imject Alum (Pierce) on days 0 and 7. Seven days later, mice were challenged on 3 consecutive days (days 14, 15 and 16) with 20 ug of DerP1 in 50 ul PBS. There were 4 mice representing this group. Negative controls included 5 mice given phosphate buffered saline (PBS) sensitization, followed by PBS challenge. In addition to 3 mice given DerP1 sensitization, followed by PBS challenge. Forty-eight hours following allergen, or control challenge whole lung tissue was harvested and total RNA was isolated.

[414] First strand cDNA was prepared using identical amounts of total RNA from each subject. IL-17F PCR was applied using Qiagen hotstar polymerase (Qiagen, Valencia, CA) and the manufacturer's recommendations. The IL-17F PCR utilized 35 cycles of amplification with sense primer, zc46098, 5' ACTTGCCATTCTGAGGGAGGTAGC 3' (SEQ ID NO:60) and antisense primer, 46099, 5' CACAGGTGCAGCCAACTTTTAGGA 3' (SEQ ID NO:61). In order to establish that the template quality was uniform amongst all subjects, Beta Actin PCR was applied to the same amount of each template used in the IL-17F amplification. B actin PCR included 25 cycles of PCR with sense primer, zc44779, 5' GTGGGCCGCTCTAGGCACCA 3' (SEQ ID NO:62) and antisense primer, zcc44776, 5' CGGTTGGCCTTAGGGTTCAGGGGGG 3' (SEQ ID NO:63).

[415] All 4 mice from the DerP1 sensitized, DerP1 challenged treatment group (the asthma simulation) showed robust IL-17F amplification. In contrast, weak IL-17F amplification was seen from the negative controls, including 3 of 3 subjects representing the DerP1 sensitized/PBS challenged treatment group and 5 of 5 subjects from the PBS sensitized/PBS challenged treatment group. B actin amplification was at least as robust for the negative controls as for the asthma-simulated subjects, demonstrating that the weak negative control IL-17F amplification was not due to template problems.

EXAMPLE 17

COS Cell Transfection and Secretion Trap

A) Cos cell transfection and secretion trap assays show that IL-17RCsR/Fc9 and IL-17F is a receptor/ligand pair

[416] A secretion trap assay was used to match the human IL-17RC (SEQ ID NO:2) to the human IL-17F (SE QID NO:16). The soluble IL-17RCsR/Fc9 fusion protein (Example 8) was used as a binding reagent in a secretion assay. SV40 ori containing expression vectors containing cDNA of human IL-17B,C,D,E, and F was transiently transfected into COS cells. The binding of IL-

17RCsR/Fc9 to transfected COS cells was carried out using the secretion trap assay described below. Positive binding of IL-17RCsR/Fc9 was only seen to human IL-17F. These results demonstrate the novel finding that human IL-17RC and IL-17F is a receptor/ligand pair.

B) COS Cell Transfections

[417] The COS cell transfection was performed as follows: Mix 3ul pooled DNA and 5ul Lipofectamine™ in 92ul serum free DMEM media (55mg sodium pyruvate, 146mg L-glutamine, 5mg transferrin, 2.5mg insulin, 1μg selenium and 5mg fetuin in 500ml DMEM), incubate at room temperature for 30 minutes and then add 400ul serum free DMEM media. Add this 500ul mixture onto 1.5x105 COS cells/well plated on 12-well tissue culture plate and incubate for 5 hours at 37°C. Add 500ul 20% FBS DMEM media (100 ml FBS, 55 mg sodium pyruvate and 146mg L-glutamine in 500ml DMEM) and incubate overnight.

C) Secretion Trap Assay

[418] The secretion trap was performed as follows: Media was rinsed off cells with PBS and then fixed for 15 minutes with 1.8% Formaldehyde in PBS. Cells were then washed with TNT (0.1M Tris-HCL, 0.15M NaCl, and 0.05% Tween-20 in H2O), and permeated with 0.1% Triton-X in PBS for 15 minutes, and again washed with TNT. Cells were blockd for 1 hour with TNB (0.1M Tris-HCL, 0.15M NaCl and 0.5% Blocking Reagent (NEN Renaissance TSA-Direct Kit) in H2O), and washed again with TNT. The cells were incubated for 1 hour with 1μg/ml human IL-17RCx1sR/FC9 soluble receptor fusion protein Cells were then washed with TNT. Cells were incubated for another hour with 1:200 diluted goat-anti-human Ig-HRP (Fc specific). Again cells were washed with TNT.

[419] Positive binding was detected with fluorescein tyramide reagent diluted 1:50 in dilution buffer (NEN kit) and incubated for 4-6 minutes, and washed with TNT. Cells were preserved with Vectashield Mounting Media (Vector Labs Burlingame, CA) diluted 1:5 in TNT. Cells were visualized using a FITC filter on fluorescent microscope.

EXAMPLE 18

Generation of Murine Anti-Human IL-17RC Monoclonal Antibodies

A. Immunization for generation of anti-IL-17RC Antibodies

1. Soluble IL-17RC-muFc

[420] Six to twelve week old intact or IL-17RC knockout mice are immunized by intraperitoneal injection with 25-50 ug of soluble human IL-17RC-muFc protein (Example 23) mixed 1:1 (v:v) with Ribi adjuvant (Sigma) on a biweekly schedule. Seven to ten days following the third immunization, blood samples were taken via retroorbital bleed, the serum harvested and evaluated for its ability to inhibit the binding of IL-17 or IL-17F to IL-17RC in neutralization assays (e.g., described herein) and to stain IL-17RC transfected versus untransfected 293 cells in a FACS staining assay.

Mice continued to be immunized and blood samples taken and evaluated as described above until neutralization titers reached a plateau. At that time, mice with the highest neutralization titers were injected intravascularly with 25-50 ug of soluble IL-17RC-Fc protein in PBS. Three days later, the spleen and lymph nodes from these mice were harvested and used for hybridoma generation, for example using mouse myeloma (P3-X63-Ag8.653.3.12.11) cells or other appropriate cell lines in the art, using standard methods known in the art (e.g., see Kearney, J.F. et al., J Immunol. 123:1548-50, 1979; and Lane, R.D. J Immunol Methods 81:223-8, 1985).

2. Soluble IL-17RC, IL-17RC-CEE, IL-17RC-CHIS, IL-17RC-CFLAG

[421] Six to twelve week old intact or IL-17RC knockout mice are immunized by intraperitoneal injection with 25-50 ug of soluble human IL-17RC-CEE, IL-17RC-CHIS, or IL-17RC-CFLAG mixed 1:1 (v:v) with Ribi adjuvant (Sigma) on a biweekly schedule. Seven to ten days following the third immunization, blood samples are taken via retroorbital bleed, the serum harvested and evaluated for its ability to inhibit the binding of IL-17 or IL-17F to IL-17RC in neutralization assays (e.g., described herein) and to stain IL-17RC transfected versus untransfected 293 cells in a FACS staining assay. Mice are continued to be immunized and blood samples taken and evaluated as described above until neutralization titers reached a plateau. At that time, mice with the highest neutralization titers are injected intravascularly with 25-50 ug of soluble IL-17RC, IL-17RC-CEE, zcytor-CHIS, or IL-17RC-CFLAG antigen protein in PBS. Three days later, the spleen and lymph nodes from these mice are harvested and used for hybridoma generation, for example using mouse myeloma (P3-X63-Ag8.653.3.12.11) cells or other appropriate cell lines in the art, using standard methods known in the art (e.g., see Kearney, J.F. et al., J Immunol. 123:1548-50, 1979; and Lane, R.D. J Immunol Methods 81:223-8, 1985).

3. P815 transfectants that express the IL-17RC

[422] Six to ten week old female DBA/2 mice are immunized by intraperitoneal injection of 1 x 105 live, transfected P815 cells, for example P815/IL-17RC cells (e.g., 0.5 ml at a cell density of 2 x 105 cells/ml). Prior to injection, the cells are maintained in the exponential growth phase. For injection the cells are harvested, washed three times with PBS and then resuspended in PBS to a density of 2 x 105 cells/ml. In this model, the mice develop an ascites tumor within 2-3 weeks and progress to death by 4-6 weeks unless an immune response to the transfected target antigen has been mounted. At three weeks mice with no apparent abdominal swelling (indicative of ascites) are re-immunized as above at 2-3 week intervals. Seven to ten days following the second immunization, blood samples are taken via retroorbital bleed, the serum harvested and evaluated for its ability to inhibit the binding of IL-17 or IL-17F to IL-17 or IL-17RC in neutralization assays (e.g., described herein) and to stain IL-17RC transfected versus untransfected 293 cells in a FACS staining assay. Mice continue to be immunized and blood samples taken and evaluated as described above until neutralization titers reach a plateau. At that time, the mice with the highest neutralization titers are

injected intraperitonealy with 1 x 105 live, transfected P815 cells. Four days later, the spleen and lymph nodes from these mice are harvested and used for hybridoma generation, for example using mouse myeloma (P3-X63-Ag8.653.3.12.11) cells or other appropriate cell lines in the art, using standard methods known in the art (e.g., see Kearney, J.F. et al., supra.; and Lane, R.D. supra.).

[423] An alternative to the above immunization scheme with live, transfected P815 cells involves intraperitoneal injection of 1-5 x 106 irradiated, transfected cells every 2-3 weeks. In this approach, no animals develop and die of ascites. Instead, animals are monitored for a neutralizing immune response to IL-17RC in their serum as outlined above, starting with a bleed after the second immunization. Once neutralization titers have reached a maximal level, the mice with highest titers are given a pre-fusion, intraperitoneal injection of 5 x 106 irradiated cells and four days later, the spleen and lymph nodes from these mice are harvested and used for hybridoma generation, for example using mouse myeloma (P3-X63-Ag8.653.3.12.11) cells or other appropriate cell lines in the art, using standard methods known in the art (e.g., see Kearney, J.F. et al., supra.; and Lane, R.D. supra.).

B. Screening the Hybridoma Fusions for Antibodies that bind IL-17RC and Inhibit the Binding of IL-17 or IL-17F to IL-17RC

[424] Three different primary screens are performed on the hybridoma supernatants at 8-10 days post-fusion. For the first assay, antibodies in supernatants were tested for their ability to bind to plate bound soluble human IL-17RC, IL-17RC-muFc, IL-17RC-CEE, IL-17RC-CHIS, or IL-17RC-CFLAG protein by ELISA using HRP-conjugated goat anti-mouse kappa and anti-lambda light chain second step reagents to identify bound mouse antibodies. To demonstrate specificity for the IL-17RC portion of the IL-17RC fusion proteins, positive supernatants in the initial assay were evaluated on an irrelevant protein fused to the same murine Fc region (mG2a), EE sequence, HIS sequence, or FLAG sequence. Antibody in those supernatants that bound to IL-17RC-fusion protein and not the irrelevant muFc or other proteins containing fusion protein sequence were deemed to be specific for IL-17RC. For the second assay, antibodies in all hybridoma supernatants were evaluated by ELISA for their ability to inhibit the binding of biotinylated human IL-17 or biotinylated human IL-17F to plate bound IL-17RC-muFc or IL-17RC-fusion proteins.

[425] All supernatants containing antibodies that bound specifically to IL-17RC, whether they inhibited the binding of IL-17 or IL-17F to IL-17RC or not in the ELISA assay, were subsequently tested for their ability to inhibit the binding of IL-17 or IL-17F to IL-17RC transfected Baf3 or BHK cells or normal human bronchial epithelial cells. All supernatants that were neutralization positive in either the IL-17 or IL-17F inhibition assays or both the IL-17 and IL-17F inhibition assays were subsequently evaluated for their ability to stain IL-17RC transfected versus non-transfected Baf3 or BHK cells by FACS analysis. This analysis was designed to confirm that inhibition of IL-17 or IL-17F binding to IL-17RC, was indeed due to an antibody that specifically

binds the IL-17RC receptor. Additionally, since the FACS analysis was performed with an anti-IgG second step reagent, specific FACS positive results indicate that the neutralizing antibody was likely to be of the IgG class. By these means, a master well was identified that bound IL-17RC in the plate bound ELISA, inhibited the binding of IL-17 or IL-17F to IL-17RC in the ELISA based inhibition assay, blocked the interaction of IL-17 and IL-17F with IL-17RC transfected Baf3 or BHK cells, respectively, and was strongly positive for the staining of IL-17RC transfected Baf3 or BHK cells with an anti-mouse IgG second step reagent.

[426] The third assay consists of primary human bronchial epithelial cells which express IL-17RC and can be induced to secrete IL-8 or IL-6 in response to IL-17F treatment. The specific monoclonal antibody is assayed by its ability to inhibit the IL-17 or IL-17F stimulated IL-8 or IL-6 production by these cells. IL-8 and IL-6 production is assayed in response to IL-17 or IL-17F as described herein.

[427] Alternatively, the monoclonal antibody; anti-IL-17RC, mediated inhibition of IL-17 or IL-17F induced luciferase production in NIH 3T3 or other IL-17RC containing cells can be used with or in place of one of the bioactivity neutralization assays noted above. The NFkB mediated luciferase assay in NIH 3T3 cells is described herein.

C) Cloning Anti-IL-17RC Specific Antibody Producing Hybridomas

[428] Hybridoma cell lines producing a specific anti-IL-17RC mAb that cross-neutralized the binding of IL-17 and IL-17F to appropriately transfected BaF3 or BHK cells are cloned by a standard low-density dilution (less than 1 cell per well) approach. Approximately 5-7 days after plating, the clones are screened by ELISA on, for example, plate bound human IL-17RC-muFc followed by a retest of positive wells by ELISA on irrelevant muFc containing fusion protein as described above.. Selected clones, whose supernatants bind to IL-17RC-muFc and not the irrelevant muFc containing fusion protein, are further confirmed for specific antibody activity by repeating both neutralization assays as well as the FACS analysis. All selected IL-17RC antibody positive clones are cloned a minimum of two times to help insure clonality and to assess stability of antibody production. Further rounds of cloning are performed and screened as described until, preferably, at least 95% of the resulting clones were positive for neutralizing anti-IL-17RC antibody production.

D) Biochemical Characterization of the Molecule Recognized by Anti-IL-17RC mAbs

[429] Biochemical confirmation that the target molecule, IL-17RC, recognized by the putative anti-IL-17RC mAbs is indeed IL-17RC are performed by standard immunoprecipitation followed by SDS-PAGE analysis or western blotting procedures, both employing soluble membrane preparations from IL-17RC transfected versus untransfected Baf3 or BHK cells. Moreover, soluble membrane preparations of non-transfected cell lines that express IL-17RC are used show that the mAbs recognize the native receptor chain as well as the transfected one. Alternatively, the mAbs are

tested for their ability to specifically immunoprecipitate or western blot the soluble IL-17RC-muFc protein.

EXAMPLE 19

Neutralization of Human IL-17RC by Sera from Mice Injected with P815 Cells Transfected with Human IL-17RC

[430] Using a cell based neutralization assay, serum from mice injected with live human IL-17RC transfected P815 cells (Example 17) is added as a serial dilution at 1%, 0.5%, 0.25%, 0.13%, 0.06%, 0.03%, 0.02%, and 0%. The assay plates are incubated at 37□C, 5% CO2 for 4 days at which time Alamar Blue (Accumed, Chicago, IL) is added at 20µl/well. Plates are again incubated at 37□C, 5% CO2 for 16 hours. Results showed that serum from four of the animals could neutralize signaling of both huIL-17 and huIL-17F through human IL-17RC.

[431] Results such as these provide additional evidence that effectively blocking IL-17RC by binding, blocking, inhibiting, reducing, antagonizing or neutralizing IL-17 or IL-17F activity (individually or together), for example via a neutralizing monoclonal antibody to IL-17RC of the present invention, could be advantageous in reducing the effects of IL-17 and IL-17F (alone or together) in vivo and may reduce IL-17 and/or IL-17F-induced inflammation, such as that seen in, for example in psoriasis, IBD, colitis, chronic obstructive pulmonary disease, cystic fibrosis or other inflammatory diseases induced by IL-17, and or IL-17F including IBD, arthritis, asthma, psoriatic arthritis, colitis, inflammatory skin conditions, and atopic dermatitis.

EXAMPLE 20

Pharmacokinetics of an Anti-human IL-17RC Monoclonal Antibody

[432] The test monoclonal antibody, anti-human IL-17RC mAb, is provided in, for example, 3x 3 mL aliquots at a concentration of approximately 1 mg/mL (determined by UV Absorbance at 280 nM) and was stored at –80 °C until use. The vehicle is 1X PBS (50mM NaPO4, 109mM NaCl), pH 7.3. The mAb is thawed at room temperature before use and aliquots 1 and 2 are used as provided for the 100 µg IV and SC dosing groups, respectively. Half of aliquot 3 is diluted 1:2 in 1X PBS for the 50 µg SC dose group and the second half of aliquot 3 is diluted 1:10 in 1X PBS for the 10 µg SC dose group. Female SCID mice (n=96) are obtained from Charles River Labs. Animals are checked for health on arrival and group-housed (3 animals per cage). The mice are 12 weeks old with an average body weight of approximately 22 g at the beginning of the study.

A) Dosing Protocol

[433] Female SCID mice (n=24/dose group) are randomly placed into four dosing groups (Table 8). Group 1 was administered the anti-human IL-17RC mAb via IV injection of approximately

93 μL in a tail vein and Groups 2, 3, and 4 are administered the mAb via SC injection of approximately 93 μL in the scruff of the neck.

B) Sample Collection

[434] Prior to blood collection, mice were fully anesthetized with halothane or isofluorane. Blood samples were collected via cardiac stick for all time points except the 168 hr timepoint (collected via eye bleed and the same animals were bled again at the 504 hr timepoint via cardiac stick). Blood was collected into serum separator tubes and allowed to clot for 15 minutes. Samples were subsequently centrifuged for 3 minutes at 14,000 rpm. Following centrifugation, aliquots of 125-150uL were dispensed into labeled eppendorf tubes and immediately stored at -80 °C until analysis.

Table 8

Group #	Dose (ROA)	Animals	PK Timepoints
1	100 μg (IV)	3 mice/timepoint*	0.25, 1, 4, 8, 24, 72, 168, 336 and 504 hr
2	100 μg (SC)	3 mice/timepoint*	0.25, 1, 4, 8, 24, 72, 168, 336 and 504 hr
3	50 μg (SC)	3 mice/timepoint*	0.25, 1, 4, 8, 24, 72, 168, 336 and 504 hr
4	10 μg (SC)	3 mice/timepoint*	0.25, 1, 4, 8, 24, 72, 168, 336 and 504 hr

* The same animals were used for the 168 and 504 hr timepoints.

C) Quantification of Serum Anti-human IL-17RC mAb Concentrations by ELISA

[435] An Enzyme Linked Immunosorbant Assay (ELISA) is developed and qualified to analyze mouse serum samples from animals dosed with anti-IL-17RC mAb during pharmacokinetic studies. This assay is designed to take advantage of a commercially available secondary antibody and colorimetric detection using TMB. The dilutions used for the standard curve were modified to improve the definition of the linear portion of the standard curve. A standard curve in the range of 100 ng/mL to 0.231 ng/mL with 2-fold dilutions allows for quantitation of the mouse serum samples. QC samples are diluted to 1:100, 1:1000 and 1:10000 in 10% SCID mouse serum and back calculated from the standard curve.

D) Pharmacokinetic Analysis

[436] Serum concentration versus time data are downloaded into WinNonlin Professional 4.0 software (Pharsight, Inc.; Cary, NC) for pharmacokinetic analysis. Noncompartmental analysis is used to determine pharmacokinetic parameters based on the mean data at each time point.

EXAMPLE 21

Neutralization of IL-17A and IL-17F Activity by a Anti- Human IL-17RC Monoclonal Antibody

[437] Using a cell-based neutralization assay, a purified mouse anti-human IL-17RC monoclonal antibody is added as a serial dilution, for example, at 10μg/ml, 5μg/ml, 2.5μg/ml, 1.25μg/ml, 625ng/ml, 313ng/ml, 156ng/ml and 78ng/ml. The assay plates are incubated at 37°C, 5% CO2 for 4 days at which time Alamar Blue (Accumed, Chicago, IL) is added at 20μl/well. Plates are again incubated at 37□C, 5% CO2 for 16 hours. This assay is able to demonstrate that the purified anti-human IL-17RC monoclonal antibody is able neutralize signaling of both huIL-17 and huIL-17F through human IL-17RC. For highly effective antibodies, when used at approx. 10μg/ml concentration, the antibody completely neutralizes proliferation induced by huIL-17 or huIL-17F, with the inhibition of proliferation decreasing in a dose dependent fashion at the lower concentrations. An isotype-matched negative control mouse mAb, tested at the concentrations described above, is exected to provide no inhibition of proliferation of either cytokine. These results are able to further demonstrate that monoclonal antibodies to IL-17RC could indeed antagonize the activity of the pro-inflammatory ligands, IL-17 and IL-17F at low concentrations.

EXAMPLE 22

IL-17A Induces Elevated Levels of IFN-gamma and TNF-alpha in Human Peripheral Blood Mononuclear Cells

[438] Human peripheral blood mononuclear cells (PBMC) are purified by ficoll density gradient centrifugation and then incubated overnight at 37oC in media alone, 50 ng/ml anti-human CD3 antibody, or the combination of 50 ng/ml anti-human CD3 antibody plus 1 □g/ml anti-human CD28 antibody. Replicate cultures for each of these conditions are set up and are given no cytokine, 25 ng/ml human IL-17A, or 25 ng/ml human IL-17F. After 24-hour incubations, supernatants from each culture are harvested and assayed for cytokine content using B-D Bioscience's human Th1/Th2 Cytometric Bead Array (CBA). We found that cultures that had been stimulated with either anti-CD3 or anti-CD3 plus anti-CD28 and had been supplemented with IL-17A contained significantly elevated levels of IFN-gamma and TNF-alpha (3-5-fold elevation of each) over cultures with no cytokine added or those that received IL-17F. Cultures in which no anti-CD3 stimulation was added did not show significant changes in cytokine levels. In addition, IL-17A addition induced no significant changes in other cytokines assayed for with the CBA including IL-2, IL-4, IL-5, and IL-10. This data indicates that IL-17A, but not IL-17F, can augment the production of IFN-gamma and TNF-alpha in PBMC cultures stimulated with anti-CD3 or anti-CD3 plus anti-CD28.

EXAMPLE 23

IL-17RC-Fc Decreases Disease Incidence and Progression in Mouse Collagen Induced Arthritis (CIA) Model

A) Mouse Collagen Induced Arthritis (CIA) Model

[439] Ten week old male DBA/1J mice (Jackson Labs) are divided into 3 groups of 13 mice/group. On day–21, animals are given an intradermal tail injection of 50-100 µl of 1mg/ml chick Type II collagen formulated in Complete Freund's Adjuvant (prepared by Chondrex, Redmond, WA), and three weeks later on Day 0 they are given the same injection except prepared in Incomplete Freund's Adjuvant. IL-17RC-Fc is administered as an intraperitoneal injection 3 times a week for 4 weeks, at different time points ranging from Day 0, to a day in which the majority of mice exhibit moderate symptoms of disease. Groups receive either 10 or 100 µg of IL-17RC-Fc per animal per dose, and control groups receive the vehicle control, PBS (Life Technologies, Rockville, MD). Animals begin to show symptoms of arthritis following the second collagen injection, with most animals developing inflammation within 1.5-3 weeks. The extent of disease is evaluated in each paw by using a caliper to measure paw thickness, and by assigning a clinical score (0-3) to each paw: 0=Normal, 0.5=Toe(s) inflamed, 1=Mild paw inflammation, 2=Moderate paw inflammation, and 3=Severe paw inflammation as detailed below.

B) Monitoring Disease

[440] Animals can begin to show signs of paw inflammation soon after the second collagen injection, and some animals may even begin to have signs of toe inflammation prior to the second collagen injection. Most animals develop arthritis within 1.5-3 weeks of the boost injection, but some may require a longer period of time. Incidence of disease in this model is typically 95-100%, and 0-2 non-responders (determined after 6 weeks of observation) are typically seen in a study using 40 animals. Note that as inflammation begins, a common transient occurrence of variable low-grade paw or toe inflammation can occur. For this reason, an animal is not considered to have established disease until marked, persistent paw swelling has developed.

[441] All animals are observed daily to assess the status of the disease in their paws, which is done by assigning a qualitative clinical score to each of the paws. Every day, each animal has its 4 paws scored according to its state of clinical disease. To determine the clinical score, the paw can be thought of as having 3 zones, the toes, the paw itself (manus or pes), and the wrist or ankle joint. The extent and severity of the inflammation relative to these zones is noted including: observation of each toe for swelling; torn nails or redness of toes; notation of any evidence of edema or redness in any of the paws; notation of any loss of fine anatomic demarcation of tendons or bones; evaluation of the wrist or ankle for any edema or redness; and notation if the inflammation extends proximally up the leg. A paw score of 1, 2, or 3 is based first on the overall impression of severity, and second on how many zones are involved. The scale used for clinical scoring is shown below.

C) Clinical Score

0 = Normal

0.5 = One or more toes involved, but only the toes are inflamed

1 = mild inflammation involving the paw (1 zone), and may include a toe or toes

2 = moderate inflammation in the paw and may include some of the toes and/or the wrist/ankle (2 zones)

3 = severe inflammation in the paw, wrist/ankle, and some or all of the toes (3 zones)

[442] Established disease is defined as a qualitative score of paw inflammation ranking 2 or more, that persists for two days in a row. Once established disease is present, the date is recorded and designated as that animal's first day with "established disease".

[443] Blood is collected throughout the experiment to monitor serum levels of anti-collagen antibodies, as well as serum immunoglobulin and cytokine levels. Serum anti-collagen antibodies correlate well with severity of disease. Animals are euthanized on Day 21, and blood collected for serum and CBC's. From each animal, one affected paw is collected in 10%NBF for histology and one is frozen in liquid nitrogen and stored at –800C for mRNA analysis. Also, 1/2 spleen, 1/2 thymus, 1/2 mesenteric lymph node, one liver lobe and the left kidney are collected in RNAlater for RNA analysis, and .1/2 spleen, 1/2 thymus, 1/2 mesenteric lymph node, the remaining liver, and the right kidney are collected in 10% NBF for histology. Serum is collected and frozen at -800C for immunoglobulin and cytokine assays.

[444] Groups of mice receiving IL-17RC-Fc at all time points are characterized by a delay in the onset and/or progression of paw inflammation. These results indicate that IL-17RC can reduce inflammation, as well as disease incidence and progression associated with this model. These results are further supported by the observation that IL-17RC-Fc resulted in decreased levels of serum TNFa, IL-1b, and anti-collagen antibodies.

EXAMPLE 24

Stable Over-Expression of IL-17RC in the Murine Assay Cell Line, Nih3t3/kz142.8 Expressing the ap1/nfkb Transcription Factor

[445] The murine nih3t3/kz142.8 assay cell line was transfected with a human IL-17RCx1 (SEQ ID NO:2) in an expression vector with a methotrexate resistance gene (dihydrofolate reductase,DHFR) This transfection was performed using a commercially available kit and the manufacturer's recommendations. (Mirus, Madison,WI. Cat. #MIR218) Cells were placed in 1μM mtx amended growth medium to select for the expression vector containing the human IL-17RCX1 transgene. After selection a human IL-17RCx1 transfection pool was generated, and called nih3t3/kz142.8/hcytor14x1.

A) Luciferase assay using the nih3t3/kz142.8 assay cell line

[446] Since nih3t3/kz142.8 has a stable kz142 reporter, there is no need for adenovirus infection to add this reporter. Thus the luciferase assay protocol was shorted and done the following way:

1. Cell plating

[447] nih3t3/kz142.8 cells were plated at 5000 cells/well in solid white, cell culture coated 96 well plates, (Cat. #3917. Costar) using DMEM/10% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02. On this second day, the plating media was removed and exchanged for DMEM/1% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02.

2. Luciferase assay measuring IL-17A and F activation of the stable kz142 reporter

[448] Following the overnight incubation in the 1% fbs, DMEM media, human IL-17A,and IL-17F ligand dilutions were made in serum free media, amended with BSA to a .28% level. After adding the ligand dilutions, cells were incubated at 37oC and 5% C02 for 4 hours, after which the media was removed, cells lysed for 15 minutes and mean fluorescence intensity (MFI) measured using the luciferase assay system and reagents, (Cat.#e1531 Promega. Madison, WI.) and a Microplate luminometer. Activity was detected for both ligands at concentrations ranging from .1-1000ng/ml. The nih3t3/kz142.8/hcytor14x1 transfection pool showed similar activity for the murine IL-17A ligand as did the parental cell line. (example 14) However, the cytor14x1 transfectant pool showed an elevated responsiveness to human IL-17A and F treatments, even when these ligand concentrations were as low as 20 femptograms. The fact that the mIL-17A signaling is comparable to that in the parental cell line (example14) suggests that there isn't a general, non-specific problem with human IL-17RC-expressing cells and that the murine IL-17A is probably signaling through the endogenous murine nih3t3 cell IL-17R or IL-17RC receptor. Thus, the fact that human IL-17A and IL-17F cause an elevation of MFI at such low ligand concentrations may indicate a specific hyper-responsiveness of the cells to those ligands, which is mediated through the over-expressed human IL-17RC receptor.

[449] This result has significant clinical and biological ramifications and utility. For example, physiological situations could cause local up-regulation of the IL-17RC receptors which could then make these areas hyper-responsive to IL-17A and IL-17F, resulting in biological activation at much lower ligand concentrations than those suggested without IL-17RC over-expression. Thus, far lower soluble receptor levels may be sufficient to antagonize these hypothetically lower ligand concentrations, than previously thought or recognized by those in the field.

EXAMPLE 25

Antagonists to IL-17F and IL-17A Activity Decrease Disease Incidence and Progression in an Inflammatory Bowel Disease (IBD) Model

[450] This model is designed to show that cultured intestinal tissue from patients with IBD produce higher levels of inflammatory mediators compared to tissue from healthy controls. This enhanced production of inflammatory mediators (including but not limited to IL-1b, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, MCP-1, G- and GM-CSF, etc.) contributes to the symptoms and pathology associated with IBDs such as Crohn's disease (CD) and ulcerative colitis (UC) by way of their effect(s) on activating inflammatory pathways and downstream effector cells. These pathways and components then lead to tissue and cell damage/destruction observed in vivo. Therefore, this model can simulate this enhanced inflammatory mediator aspect of IBD. Furthermore, when intestinal tissue from healthy controls or from human intestinal epithelial cell (IEC) lines is cultured in the presence of these inflammatory components, inflammatory pathway signaling can be observed, as well as evidence of tissue and cell damage.

[451] Therapeutics that would be efficacious in human IBD in vivo would work in the above ex vivo or IEC models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators.

[452] In this model, human intestinal tissue is collected from patients with IBD or from healthy controls undergoing intestinal biopsy, re-sectioning or from post-mortem tissue collection, and processed using a modification of Alexakis et al (Gut 53:85-90; 2004). Under aseptic conditions, samples are gently cleaned with copious amounts of PBS, followed by culturing of minced sections of tissue, in the presence of complete tissue culture media (plus antibiotics to prevent bacterial overgrowth). Samples from the same pool of minced tissue are treated with one of the following: vehicle (PBS); recombinant human (rh) IL-17A; rhIL-17F; or rhIL-17A+rhIL-17F. In addition, these are treated with or without an antagonist of either IL-17A or IL-17F, alone or in combination (such as a soluble IL-17RC). This experimental protocol is followed for studies with human IEC lines, with the exception that cells are passaged from existing stocks. After varying times in culture (from 1 h to several days), supernatants are collected and analyzed for levels of inflammatory mediators, including those listed above. In samples from patients with IBD or in samples treated with rhIL-17A and/or F, levels of inflammatory cytokines and chemokines are elevated compared to untreated healthy control tissue samples. The addition of antagonists to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention markedly reduces the production of inflammatory mediators, and thus, would expect to be efficacious in human IBD.

EXAMPLE 26

Antagonists to IL-17F and IL-17A activity Decrease Disease Incidence and Progression in a Multiple Sclerosis (MS) Model

[453] Multiple sclerosis (MS) is a complex disease that is thought to be mediated by a number of factors, including the presence of lymphocytic and mononuclear cell inflammatory infiltrates and demyelination throughout the CNS. Microglia are macrophage-like cells that populate the central nervous system (CNS) and become activated upon injury or infection. Microglia have been implicated as playing critical roles in various CNS diseases including MS, and may be used to study mechanism(s) of initiation, progression, and therapy of the disease (Nagai et al. Neurobiol Dis 8:1057-1068; 2001; Olson et al. J Neurosci Methods 128:33-43; 2003). Immortalized human microglial cell lines and/or established human astroglia cell lines can, therefore, be used to study some of the effects of inflammatory mediators on these cell types and their potential for neutralization. Inflammatory mediators (including but not limited to IL-1b, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, RANTES, IP-10, MCP-1, G- and GM-CSF, etc.) can contribute to the symptoms and pathology associated with MS by way of their effect(s) on activating inflammatory pathways and downstream effector cells.

[454] In order to evaluate the pro-inflammatory actions of IL-17A and IL-17F, and the ability of an antagonist to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention to neutralize or decrease these effects, cultured glial cells are treated with one of the following: vehicle; rhIL-17A; rhIL-17F; rhIL-17A+IL-17F. In addition, these are treated with or without an antagonist of either IL-17A or IL-17F, alone or in combination (such as a soluble IL-17RC). After varying times in culture (from 1 h to several days), supernatants and cells are collected and analyzed for levels and/or expression of inflammatory mediators, including those listed above. Levels of inflammatory cytokines and chemokines are elevated in the presence of rhIL-17A and/or IL-17F compared to cultures treated with vehicle alone. The addition of antagonists to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention markedly reduces the production and expression of inflammatory mediators, and thus, would expect to be efficacious in inflammatory aspects associated with human MS.

EXAMPLE 27

Antagonists to IL-17F and IL-17A activity Decrease Disease Incidence and Progression in a Rheumatoid Arthritis (RA) and Osteoarthritis (OA) Model

[455] This model is designed to show that human synovial cultures (including synovial macrophages, synovial fibroblasts, and articular chondrocytes) and explants from patients with RA

and OA produce higher levels of inflammatory mediators compared to cultures/explants from healthy controls. This enhanced production of inflammatory mediators (including but not limited to oncostatin M, IL-1b, IL-6, IL-8, IL-12, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, IP-10, RANTES, RANKL, MIP family members, MCP-1, G- and GM-CSF, nitric oxide, etc.) contributes to the symptoms and pathology associated with RA and OA by way of their effect(s) on activating inflammatory pathways and downstream effector cells. These pathways and components then lead to inflammatory infiltrates, cartilage and matrix loss/destruction, bone loss, and upregulation of prostaglandins and cyclooxygenases. Therefore, this model can simulate the destructive inflammatory aspects of RA and OA in in vitro and ex vivo experiments. Furthermore, when explants and synovial cultures from healthy controls are cultured in the presence of several of these inflammatory components (e.g. oncostatin M, TNF-a, IL-1b, IL-6, IL-17A and F, IL-15, etc.), inflammatory pathway signaling can be observed. Therapeutics that would be efficacious in human RA in vivo would work in the above in vitro and ex vivo models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators.

[456] In this model, human synovial explants are collected from patients with RA, OA, or from healthy controls undergoing joint replacement or from post-mortem tissue collection, and processed using a modification of Wooley and Tetlow (Arthritis Res 2: 65-70; 2000) and van 't Hof et al (Rheumatology 39:1004-1008; 2000). Cultures of synovial fibroblasts, synovial macrophages and articular chondrocytes are also studied. Replicate samples are treated with one of the following: vehicle (PBS); recombinant human (rh) IL-17A; rhIL-17F; or rhIL-17A+rhIL-17F, and some samples contain various combinations of oncostatin M, TNF-a, IL-1b, IL-6, IL-17A, IL-17F, and IL-15. In addition, these are treated with or without an antagonist to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention. After varying time of culture (from 1 h to several days), supernatants are collected and analyzed for levels of inflammatory mediators, including those listed above. In samples from patients with RA or OA, or in samples treated with rhIL-17A and/or F (either alone or in combination with other inflammatory cytokines), levels of inflammatory cytokines and chemokines are elevated compared to untreated healthy control explants or in untreated cell cultures. The addition of antagonists to IL-17F and/or IL-17A activity, such as IL-17RC soluble receptors and antibodies thereto including the anti-human-IL-17RC monoclonal and neutralizing antibodies of the present invention markedly reduces the production of inflammatory mediators, and thus, would expect to be efficacious in human RA and OA.

EXAMPLE 28

IL-17A and IL-17F Functional Responses

[457] NIH-3T3/KZ142 cells were stably transfected with human IL-17RCx1 (SEQ ID NO:1) and mouse IL-17RCx1 (SEQ ID NO:25). As described above, each line was treated for 7 and 15 minutes with a dose response of IL-17A, IL-17F, murine IL-17F, and appropriate controls. Both IL-17A and IL-17F gave a dose dependent response in phosphorylated IκB-α and p38 MAPK transcription factors when IL-17RCx1 (SEQ ID NO:1) was transfected, approximately 30% greater then the inherent signaling from the control line. IL-17A and IL-17F gave no increase in signaling when the murime IL-17RCx1 (SEQ ID NO:25) was transfected. Murine IL-17F gave no increase in signaling for either human or murine IL-17RCx1.

EXAMPLE 29

IL-17A, IL-17F, IL-17RA and IL-17RC Expression in Murine Disease Models

[458] Four murine models of disease (asthma, DSS colitis, atopic dermatitis and experimental allergic encephalomyelitis) were analyzed using know techniques for the expression of IL-17A, IL-17F, IL-17R and IL-17RC.

[459] In the asthma model, IL-17A and IL-17F are expressed at very low to undetectable levels in lung, spleen, lung draining lymph nodes and lung infiltrating cells in diseased and non-diseased mice. IL-17RC message was found to be more highly expressed in lung compared to spleen and lymph node but was not regulated with disease. IL-17R was more highly expressed in spleen and lung draining lymph node compared to lung but was also not regulated with disease.

[460] Contrary to the asthma model, IL-17A and IL-17F were highly up-regulated in diseased but not normal mice in the DSS-colitis model in both proximal and distal colon. Neither cytokine was significantly up-regulated in the mesenteric lymph node. Further, it was found that up-regulation of both cytokines in the context of acute DSS-induced colitis and not in chronic DSS-induced colitis. IL-17R was found to be prominently expressed in mesenteric lymph nodes as compared to proximal and distal colon, but was not regulated with disease. In contrast, IL-17RC was more highly expressed in proximal distal colon tissue compared to mesenteric lymph nodes. IL-17RC expression was also not regulated with disease.

[461] In atopic dermatitis, IL-17A mRNA was not detectable. IL-17F was found to be expressed in both skin and skin-draining lymph nodes but did not appear to be significantly regulated with disease. IL-17R mRNA was more highly expressed in skin-draining lymph nodes as compared to skin but was not regulated with disease. IL-17RC was more highly expressed in skin compared to skin-draining lymph nodes but was also not regulated with disease.

[462] In experimental allergic encephalomyelitis, both IL-17A and IL-17F appeared to up-regulated in spinal chord in diseased but not healthy mice. IL-17F may have been more highly

expressed in lymph nodes compared to spinal cord but expression in the lymph nodes was not regulated with disease. However, overall levels of expression in these tissues was quite low. IL-17R was more highly expressed in lymph node tissue compared to brain and spinal cord. IL-17RC was not tested.

[463] In short, IL-17A and IL-17F expression appears to be regulated with disease in the context of the DSS-induced colitis and experimental allergic encephalomyelitis models but apparently not for asthma or atopic dermatitis. IL-17R and IL-17RC expression does not appear to be regulated with disease but IL-17R expression appears to be enriched in lymphoid tissues while IL-17RC expression appears to be enriched in non-lymphoid tissues.

EXAMPLE 30

IL-17RC is a Mediator of Activation to Both IL-17A and IL-17F

[464] The murine nih3t3/kz142.8 assay cell line was transfected with a human IL-17RCX1 (SEQ ID NO:2) in an expression vector with a methotrexate resistance gene. (dihydrofolate reductase,DHFR) Human IL-17RA (SEQ ID NO:21) was similarly tranfected into this cell line. Transfections were performed using a commercially available kit and the manufacturer's recommendations. (Mirus, Madison,WI. Cat. #MIR218) Cells were placed in 1μM mtx amended growth medium to select for the expression vector containing the expression constructs. After selection transfection pools were generated, and called nih3t3/kz142.8/hcytor14X1 and nih3t3/kz142.8/IL-17R.

A) Luciferase assay using the nih3t3/kz142.8- based cell lines.

[465] Since nih3t3/kz142.8 based cell lines have stable ap1/nfkb reporters (kz142), there is no need for adenovirus infection to add this reporter. Thus the luciferase assay protocol was shorted and done the following way:

1. Cell plating

[466] Cells were plated at 5000 cells/well in solid white, cell culture coated 96 well plates, (Cat. #3917. Costar) using DMEM/10% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02. On this second day, the plating media was removed and exchanged for DMEM/1% FBS, containing glutamine and amended with pyruvate and cultured overnight at 37oC and 5% C02.

2. Luciferase assay measuring IL-17A and F activation of the stable kz142 reporter

[467] Following the overnight incubation in the 1% fbs, DMEM media, human IL-17A,and IL-17F ligand dilutions were made in serum free media, amended with BSA to a .28% level. After adding the ligand dilutions, cells were incubated at 37oC and 5% C02 for 4 hours, after which the media was removed, cells lysed for 15 minutes and mean fluorescence intensity (MFI) measured using the luciferase assay system and reagents, (Cat.#e1531 Promega. Madison, WI.) and a

Microplate luminometer. Activity was detected for both ligands at concentrations ranging from .1-100ng/ml.

[468] The EC50s discussed below are averages of at least 4 experiments. The nih3t3/kz142.8/hcytor14x1 transfection pool showed similar activity for the murine IL-17A ligand as did the parental cell line, with an EC50 of about 4ng/ml. (example 14) The fact that the mIL-17A signaling in the hcytor14x1 recombinant line is comparable to that in the parental cell line (example14) suggests that murine IL-17A is probably signaling through the endogenous murine nih3t3 cell IL-17RA or IL-17RC receptors and does not activate the cells through hcytor14X1. However, the hIL-17RCX1 transfectant pool showed an elevated responsiveness to human IL-17A treatment, with an EC50 of .41 ng/ml Vs 2.8 ng/ml (averages of 4 experiments) in the parental line (a 6.8 fold more potent EC50 in the recombinant line) In addition, the hIL-17RCX1 recombinant line had an enhanced responsiveness to hIL-17F, with an EC50 of .61ng/ml in the recombinant line Vs 10ng/ml in the parental line. (a 17 fold more potent EC50 in the recombinant line). The increased potency to hIL-17A and F in the hIL-17RCX1 line is consistent with human IL-17RCX1 being a high affinity receptor for both human IL-17A and IL-17F. In contrast, the hIL-17RA recombinant line had enhanced sensitivity only to hIL-17A, with an EC50 of .6ng/ml vs 2.8 ng/ml for the parental line. There was not an enhancement of the hIL-17F EC50 in the hIL-17RA recombinant line, with an IL-17F EC50 of 12.4 ng/ml vs 8.9ng/ml in the parental line.

[469] This result is significant because it specifically implicates hIL-17RCX1 as a mediator of activation to both hIL-17A and hIL-17F and suggests that hIL-17RA mediates signaling only to hIL-17A activation and not hIL-17F.

EXAMPLE 31

Intravenous Administration of IL-17A and IL-17F

[470] To determine the effect of i.v. delivery of murine or human IL-17A or IL-17F on complete blood counts (CBC) and serum cytokines/chemokines in BALB/c mice at various time points.

[471] I.V. administration of 1 ug mIL-17A resulted in an approximate 2-fold increase in circulating neutrophils (by CBC) and approximate 10-fold increase in serum KC and MCP-1 (by Luminex) 1-2 h following administration; similar results in these chemokines were observed with 5 ug hIL-17A. Blood monocyte levels were also significantly increased in mice treated with 1 ug mIL-17A (showed the greatest increase), 5 ug hIL-17A or 5 ug hIL-17F at the 2 h timepoint. I.V. administration of m and hIL-17F resulted in marked increases in serum IL-15 (by Luminex) at the 1 and 2 h time points, and small increases in serum KC and MCP-1 at these same timepoints.

EXAMPLE 32

Neutralization of Intravenous Administration IL-17A and IL-17F

[472] To neutralize the i.v. IL-17A and IL-17F-mediated increases in cytokines and chemokines with i.p. soluble receptors (mIL-17RA:Fc for murine ligands; soluble human IL-17RC for human ligands). Female BALB/c mice were administered by i.p. injection either PBS, 100 ug mIL-17RA:Fc, or 100 ug soluble human IL-17RC three hours prior to receiving by i.v. tail injection: PBS; 2 ug of either mIL-17A, mIL-17F, or 2 ug of both mIL-17A and F (for mice that received mIL-17RA:Fc); or 2 ug of either hIL-17A, hIL-17F, or 2 ug of both hIL-17A and F (for mice that received soluble human IL-17RC). Serum was collected 1 h following ligand administration and analyzed for a small number of serum cytokines and chemokines.

[473] Mice pretreated with i.p. soluble receptor had marked reductions in IL-17A-mediated increases in serum concentrations of IL-17A and KC compared to mice treated with PBS +IL-17A.

EXAMPLE 33

Plate Based Protein binding Assays of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides

[474] The format of the Capture EIA is as follows: Coat the ELISA plate with Goat anti Human IgG at 1 μg/ml and incubate overnight at 4°C. Wash and block the plate with 200 μl per well 1% BSA for 1 hour at room temperature. Wash, add the soluble receptor variants (A1586F, A1587F) or IL17RCx1 (A1034F) dilution series (100 μg/ml through 0.10 μg/ml) to the plate and incubate for 1 hour at room temperature. Wash, add biotin labeled ligand @ 10:1 (IL17A) or 6:1 (IL17F) and incubate for 1 hour at room temperature. Wash, add Strept Avidin –Horse Radish Peroxidase @ 0.5 μg/mL and incubate for 1 hour at room temperature. Wash, add TMB substrate for 4 minutes. Stop the reaction by adding Stop Solution. (Note: All reagents volumes were 50 μl per well unless stated otherwise). A positive result would be high OD values, generally above 0.5. The results indicated that construct 1342 (SEQ ID NO:74) does not bind IL-17A and weakly binds IL-17F in this assay. Construct 1341 (SEQ ID NO:72) binds both IL-17A and IL-17F very strongly. IL-17RCx1 binds IL-17A and IL-17F.

[475] The format of the Neutralization EIA is as follows: Coat the ELISA plate with soluble receptor (A1034F) at 1 μg/ml and incubate overnight at 4°C. Wash and block the plate with 200 μl per well 1% BSA for 1 hour at room temperature. While blocking, in a separate plate incubate the soluble receptor variants (A1586F, A1587F) dilution series (50 μg/ml through 0.05 μg/ml) with biotin labeled ligand @ 10:1 (IL17A) or 6:1 (IL17F) in equal volumes for 1 hour at room temperature. Wash the blocked plate, add the receptor-ligand complex to the blocked plate and incubate for 1 hour at room temperature. Wash, add Strept Avidin –Horse Radish Peroxidase @ 0.5

μg/mL and incubate for 1 hour at room temperature. Wash, add TMB substrate for 7 minutes. Stop the reaction by adding Stop Solution. (Note: All reagents volumes were 50 μl per well unless stated otherwise). A positive result would be low OD values, generally below 0.5. The results indicated that construct 1342 (SEQ ID NO:74) weakly neutralizes binding of IL17A to IL17RCx1and strongly neutralizes binding of IL17F to IL17RCx1. Construct 1341 (SEQ ID NO:72) weakly neutralizes binding of IL17A to IL17RCx1 and weakly neutralizes binding of IL17F to IL17RCx1. Neutalization indicates that the variant protein is binding the biotinylated ligand.

EXAMPLE 34

FACS Binding Assay Protocol

[476] To assess the ability of the soluble IL-17RC and IL-17RC/IL-17RA polypeptides of the present invention to bind the ligands IL-17A and IL-17F, a Flow Cytometry-based competitive binding assay was utilized. Incubation of a BHK cell line stably transfected with full length IL17RCx4 in the presence of the ligands IL17A or IL17F, and the soluble receptor targeted to bind the ligands allows for detection and relative quantification of ligand bound to the cell surface (and therefore unbound by the soluble receptor). The biotinylation of the ligand allows for FACS detection using a secondary Streptavidin conjugated fluorophore. A reduction in cell bound ligand over a titration of the soluble receptor is recorded as a reduction in the mean fluorescence of the cells. Biotinylated ligands are individually pre-mixed at 1ug/ml with titrating amounts of soluble receptor in staining media (HBSS + 1%BSA + 0.1% NaAzide + 10mM HEPES) in 100ul volumes and incubated at RT for 15 minutes. A BHK cell line stably transfected with full length IL17RCx4 is prepared for ligand staining by resuspension with Versene (Invitrogen cat.15040-066), equilibrating to 2 x 10e5 cells/100ul, pelleting, and resuspension in the ligand/soluble receptor pre-mix. Stained cells are incubated at 4° for 30 minutes, washed 1x in staining media, and stained with Streptavidin-PE (BD Pharmingen cat. 554061) at a 1:100 ratio. Cells are incubated at 4° in the dark for 30 minutes, washed 2x in staining media, and re-suspended in a 1:1 ratio of staining media and Cytofix (BD Bioscience 554655). The BD LSRII Flow Cytometer or similar instrument is used for data collection and analysis. Figure 5 depicts a standard graph. The graph was generated using the Prizm software program. The Y values represent the MFI normalized to maximum and minimum (100% and 0%) based on ligand only and no ligand/no soluble receptor control wells, and thus the percent binding of the ligand to the cells. The software calculates the IC50 for each curve.

EXAMPLE 35

Inhibition of Specific Binding of Biotinylated Human IL-17A and IL17F with a Soluble IL-17RC/IL-17RA Polypeptide

[477] The binding assay used to determine the ability of the soluble IL-17RC and IL-17RC/IL-17RA polypeptides to bind IL-17A and IL17F is described herein. Binding studies are performed as discussed above, except that additional soluble polypeptides, such as SEQ ID NOs: 157 and 158 was included in the binding reaction. This soluble polypeptide inhibited binding of both human IL-17A and IL-17F to IL-17RC transfected BHK cells to the same extent as soluble human IL-17RCx1 Fc fusion protein (SEQ ID NO:64). The remainder of soluble polypeptides, including the soluble polypeptide of SEQ ID Nos: 157 and 158, are included in Table 9 below.

Table 9*

Soluble Polypeptide	Variant	IC50 - IL17A		Soluble Polypeptide	Variant	IC50 - IL17F
IL17RA/RC	1407	7		IL17RC	1390	9
IL17RA/RC	1407	9		IL17RA/RC	1454	18
IL17RA/RC	1454	4		IL17RA/RC	1454	31
IL17RA/RC	1454	17		IL17RA/RC	1454	95
IL17RA/RC	1454	20		IL17RA/RC	1407	33
IL17RC	1390	12		IL17RA/RC	1407	42
IL17RA/RC	1341	30		IL17RC	1210	31
IL17RC	1210	35		IL17RC	1210	61
IL17RC	1210	47		IL17RC	1210	67
IL17RC	1210	74		IL17RA/RC	1341	47
IL17RC	1459	126		IL17RC	1459	103
IL17RC	1342	217		IL17RC	1342	313

* Cell-based Competition Binding IC50 (ng/uL); ordering of Constructs from strongest binders to weakest based on IC50's for each ligand

EXAMPLE 36

Binding Affinity of the IL-17RC and IL-17RC/IL-17RA Soluble Polypeptides to IL-17A and IL-17F

[478] IL-17RCx1, IL-17RA and the soluble IL-17RC/IL-17RA soluble polypeptide (SEQ ID Nos: 157 and 158) were tested for binding affinity to both IL-17A and IL-17F as follows: Gt-anti-Hu IgG-Fc specific Antibody (Jackson #109-005-008) was diluted to 50ug/ml in pH 5.0 Na Acetate and immobilized onto a CM5 Biacore chip. The protocol was optimized to capture receptor at a

theoretical binding max. before injecting a concentration series of each ligand to observe association and dissociation. The soluble receptors and the IL-17RC/IL-17RA polypeptide were tested for binding of a concentration series of each ligand. The surface was regenerated with 2 x 30 sec. injections of pH 1.75 glycine between cycles. Data was evaluated using Biacore Evaluation software to define kinetic values and is hown in Table 10 below.

Table 10*

Human IL17RCx1 Affinity for Human IL-17A 05-2005

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
1.05E+06	4.90E-04	4.69E-10	9.02	0.424
1.24E+06	4.38E-04	3.52E-10	8.86	0.324

Human IL17RCx1 Affinity for Human IL-17F 05-2005

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
9.91E+05	4.31E-04	4.35E-10	7.22	0.378
1.11E+06	3.84E-04	3.46E-10	7.57	0.549

Soluble IL-17RC/IL-17RA Polypeptide for Human IL-17A 04-2006

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
1.42E+06	6.22E-05	4.39E-11	20.5	0.460
2.61E+06	9.95E-05	3.82E-11	18.3	0.888

Soluble IL-17RC/IL-17RA Polypeptide for Human IL-17F 04-2006

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
1.82E+06	2.61E-04	1.43E-10	10.2	0.495
2.49E+06	3.15E-04	1.26E-10	11.2	0.544

Human IL-17RA Affinity for Human IL-17A 06-2006

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
3.70E+05	8.65E-05	2.34E-10	29.5	0.249
2.89E+05	8.57E-05	2.96E-10	35.1	0.197

Human IL-17RA Affinity for Human IL-17F 07-2006

ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
2.09E+04	5.56E-04	2.66E-08	20.3	0.071
2.55E+04	4.40E-04	1.72E-08	9.9	0.076

*Equilibrium and rate constants are shown and values fall within machine limits.

Chi2 refers to the sum of the square of the residuals between the binding curves and the evaluation fitting curves. The closer to 0, the more confidence we have in the data. This data is shown with good confidence.

[479] These data demonstrates the binding of human IL-17A and human IL-17F to human IL-17RA and human IL-17RC. Specifically, human IL-17RC demonstrates similar binding affinity for both human IL-17A and human IL-17F with dissociation equilibrium constants (KD) in the 400 picomolar (pM) range. The soluble IL-17RC/IL-17RA polypeptide bound human IL-17A with slightly higher affinity, KD~ 40pM, than human IL-17F, KD~ 140pM. Human IL-17RA produced the largest discrepancy of ligand affinity with a 100-fold difference between human IL-17A, KD~ 300pM, and human IL-17F, KD~ 30 nanomolar (nM),binding.

EXAMPLE 37

Creation of Recombinant Human IL-17RA/NIH3T3/KZ142.8 and IL-17RCx4/NIH3T3/KZ142.8 Reporter Assay Cell Lines

[480] The murine NIH3T3/KZ142.8 reporter cell line described herein was used to create new assay cell lines, recombinant for either human IL-17RA (SEQ ID NO:21) or IL-17RCx4 (SEQ ID NO:166). This was accomplished by transfection of these cells with expression constructions containing each of these cDNAs. The expression vector utilized, pzmp11, which contains the dihydrofolate reductase gene. Thus transfectants were selected using 1uM methotrexate amended growth medium to create stable pools. These assay cell lines were called hIL-17RA/NIH3T3/KZ142.8 and hIL-17RCX4/NIH3T3/KZ142.8.

EXAMPLE 38

A Soluble IL-17RC/IL-17RA Polypeptde Antagonizes Human IL-17A Activation of Recombinant Human IL-17RA/NIH3T3/KZ142.8 Cells

[481] The efficacy of soluble IL-17RC/IL-17RA soluble polypeptide (SEQ ID Nos: 157 and 158) competition for human IL-17A activation of recombinant hIL-17RA/NIH3T3/KZ142.8 cells was measured as follows: Cell plating and preparation for a luciferase assay was the same as that described herein. The day of the assay, these cells were first given a triplicate 2 fold dose series of one volume of soluble receptors at 2 fold the final concentration including the soluble polypeptide above, IL-17RA and IL-17RC beginning at a 2ug/ml, (which results in a 1ug/ml final concentration once combined with the ligand). Next one volume of IL-17A was applied at 1ng/ml, which is 2 fold the final concentration of .5ng/ml which results from the receptor-ligands mixing together. The maximum activation was determined using a triplicate set which received .5ng/ml of IL-17A without receptor. The basal activation was determined using a triplicate set which received only assay medium which contained neither ligand nor soluble receptor. Data analysis revealed IC50 for IL-17A

activation of the above cell line by the soluble polypeptide was 7ng/ml. There wasn't sufficient potency of soluble IL-17RA or IL-17RC to convincingly antagonize .5ng/ml hIL-17A activation of this cell line with even the highest dose of 1ug/ml soluble receptor.

EXAMPLE 39

A Soluble IL-17RC/IL-17RA Polypeptide Antagonizes Human IL-17F Activation of Recombinant Human IL-17RA/NIH3T3/KZ142.8 cells

[482] The efficacy of the soluble IL-17RC/IL-17RA polypeptide (SEQ ID Nos: 157 and 158) competition for human IL-17F activation of recombinant hIL-17RA/NIH3T3/KZ142.8 cells (described above) was measured as follows: Cell plating and preparation for a luciferase assay was the same as that described herein. The day of the assay, these cells were first given a triplicate 2 fold dose series of one volume of soluble polypeptide at 2 fold the final concentration including the soluble polypeptide above, IL-17RA and IL-17RC beginning at a 4ug/ml, (which results in a 2ug/ml final concentration once combined with the ligand). Next one volume of IL-17F was applied at 40ng/ml, which is 2 fold the final concentration of 20ng/ml which results from the receptor-ligands mixing together. The maximum activation was determined using a triplicate set which received 20ng/ml of IL-17F without receptor. The basal activation was determined using a triplicate set which received only assay medium which contained neither ligand nor soluble receptor. Data analysis revealed IC50 for IL-17F activation of the above cell line by the IL-17RC/IL-17RA soluble polypeptide of 0.48ug/ml. There wasn't sufficient potency of soluble IL-17RA or IL-17RC to show any antagonism of 20ng/ml IL-17F activation of this cell line with even the highest dose of 2ug/ml soluble receptor.

EXAMPLE 40

A Soluble IL-17RC/IL-17RA Polypeptide Antagonizes Human IL-17F Activation of Recombinant Human IL-17RCx4/NIH3T3/KZ142.8 cells

[483] The efficacy of soluble IL-17RC/IL-17RA polypeptide (SEQ ID Nos: 157 and 158) competition for IL-17F activation of recombinant hIL-17RCX4/NIH3T3/KZ142.8 cells (described above) was measured as follows: Cell plating and preparation for a luciferase assay was the same as that described herein. The day of the assay, these cells were first given triplicate 5 fold serial doses of one volume of soluble receptors at 2 fold the final concentration including the above soluble polypeptide, IL-17RA and IL-17RC beginning at a 4ug/ml. Next one volume of IL-17F lot A1275F was applied at 2ng/ml, which is 2 fold the final concentration of 1ng/ml which results from the receptor-ligands mixing together. The maximum activation was determined using a triplicate set which received 1ng/ml of IL-17F without receptor. The basal activation was determined using a triplicate set which received only assay medium which contained neither ligand nor soluble receptor.

Data analysis revealed IC50 for IL-17F activation of the soluble IL-17RC/IL-17RA polypeptide of 0.8ug/ml, IL-17RC was 6ug/ml, and IL-17RA had no antagonism at any dose.

EXAMPLE 41

Soluble IL-17RC/IL-17RA Polypeptide Neutralizes the Activity of Both Human IL-17A and IL-17F Induction of G-CSF, IL-6 and IL-8

[484] Human small airway epithelial cells (SAEC) were treated with human IL-17A or with human IL-17F and 48hr supernatants were collected. These supernatants were assayed and showed a dose-dependent induction of G-CSF, IL-6, and IL-8, as shown in Table 11 below:

Table 11

	Fold Induction in 48hr supernatants		
	G-CSF	IL-6	IL-8
SAEC treated with:			
huIL-17A 50 ng/ml	26	13	8
10 ng/ml	24	14	6
2 ng/ml	14	8	3
0.4 ng/ml	13	8	3
huIL-17F 250 ng/ml	15	11	4
50 ng/ml	10	8	3
10 ng/ml	8	8	2
2 ng/ml	4	5	2

[485] SAEC were also treated with 0.01 – 10 ug/ml doses of soluble IL-17RC/IL-17RA polypeptide (SEQ ID Nos: 157 and 158) in combination with 10 ng/ml human IL-17A or 50 ng/ml human IL-17F (both ligand and soluble polypeptide were incubated together for 30 minutes at 37^{0}C before adding to cells), and 48hr supernatants collected. As shown in Table 12 below, these supernatants showed decreased G-CSF, IL-6, and IL-8, demonstrating that the soluble IL-17RC/IL-17RA polypeptide was able to effectively neutralize the activity of both human IL-17A and human IL-17F induction of these cytokines. It is noted that IC50 values were not able to be determined for the neutralization of IL-6, because at the lowest dose (0.01 ug/ml) of the soluble IL-17RC/IL-17RA polypeptide tested, neutralization had only returned to approximately 50% of max.).

Table 12

Soluble IL-17RA/RC receptor neutralizes activity of huIL-17A/F :	IC50 of IL-17RA/RC (ug/ml)
huIL-17A(10 ng/ml) induction of G-CSF	0.14
huIL-17F(50 ng/ml) induction of G-CSF	1.20
huIL-17A(10 ng/ml) induction of IL-8	0.03
huIL-17F(50 ng/ml) induction of IL-8	0.57
huIL-17A(10 ng/ml) induction of IL-6	94% neutralized at 10 ug/ml 49% neutralized at 0.01 ug/ml
huIL-17F(50 ng/ml) induction of IL-6	72% neutralized at 10 ug/ml 57% neutralized at 0.01 ug/ml

EXAMPLE 42

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Human Multiple Sclerosis Samples

[486] Multiple sclerosis (MS) is a complex disease that is thought to be mediated by a number of factors, including the presence of lymphocytic and mononuclear cell inflammatory infiltrates and demyelination throughout the CNS. Microglia are macrophage-like cells that populate the central nervous system (CNS) and become activated upon injury or infection. Microglia and neuronal cells have both been implicated as playing critical roles in various CNS diseases including MS, and may be used to study mechanism(s) of initiation, progression, and therapy of the disease (Nagai et al. Neurobiol Dis 8:1057-1068; 2001; Olson et al. J Neurosci Methods 128:33-43; 2003; Giuliani et al. J Neuroimmunol 165: 83 – 91; 2005). Primary neuronal cell cultures, immortalized human microglial cell lines and/or established human astroglia cell lines can, therefore, be used to study some of the effects of inflammatory mediators on these cell types and their potential for neutralization. Inflammatory mediators (including but not limited to IL-1b, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, RANTES, IP-10, MCP-1, G- and GM-CSF, etc.) can contribute to the symptoms and pathology associated with MS by way of their effect(s) on activating inflammatory pathways and downstream effector cells.

[487] In order to evaluate the pro-inflammatory actions of IL-17A and IL-17F on these cells types, and the ability of the soluble polypeptides of the present invention, such as the soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) to neutralize or decrease these effects, cultured neuronal or glial cells are treated with one of the following: vehicle; rhIL-17A; rhIL-17F; rhIL-17A+IL-17F. In addition, these are treated with or without a soluble polypeptide of the present invention, such as the soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158). In a separate set of

cultures, circulating T cells isolated from human subjects and activated with anti-CD3, are added to the cultured neuronal and glial cells in the absence of exogenous IL-17A or IL17-F, thus providing a co-culture method of investigating the destructive effects of activated T cells on these cell types. The T cells are treated with or without a soluble polypeptide of the present invention, such as the soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158). After varying times in culture (from 1 h to several days), supernatants and cells are collected and analyzed for levels and/or expression of inflammatory mediators, including those listed above, and also analyzed for cell survival. Levels of inflammatory cytokines and chemokines, and death of neuronal cells, are elevated in the presence of rhIL-17A and/or IL-17F compared to cultures treated with vehicle alone. The addition of a soluble polypeptide of the present invention, such as the soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) markedly reduces the production and expression of inflammatory mediators in these cultures, and increases cell survival in the neuronal cells.

[488] Therefore, because these *ex vivo* experiments demonstrate that a soluble polypeptide of the present invention, such as the soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) can reduce the destructive and inflammatory actions that are associated with the pathobiology of human MS, treatment with such soluble polypeptides would be expected to be efficacious in reducing the inflammatory aspects, neuronal death, and/or demyelination associated with human MS.

EXAMPLE 43

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Human Rheumatoid Arthritis ("RA") and Osteoartritis ("OA") Samples

[489] These models are designed to show that human synovial cultures (including synovial macrophages, synovial fibroblasts, and articular chondrocytes) and explants from patients with RA and OA produce higher levels of inflammatory mediators compared to cultures/explants from healthy controls, which in turn can contribute to the degradation of extracellular matrix components (e.g. bone, cartilage, etc), which is a hallmark of these diseases. In addition, the co-culture models described below are designed to show that inflammatory mediators present in RA/OA synovial fluid and/or activated T cells can also result in greater inflammation and matrix degradation.

[490] The enhanced production of inflammatory mediators (including but not limited to oncostatin M, IL-1b, IL-6, IL-8, IL-12, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, IP-10, RANTES, RANKL, MIP family members, MCP-1, MMP-9, G- and GM-CSF, nitric oxide, etc.) contributes to the symptoms and pathology associated with RA and OA by way of their effect(s) on activating inflammatory pathways and downstream effector cells. These pathways and components then lead to inflammatory infiltrates, cartilage and matrix loss/destruction, bone loss, and upregulation

of matrix metalloproteases, prostaglandins and cyclooxygenases. Therefore, these models can simulate the destructive inflammatory aspects of RA and OA in *in vitro* and *ex vivo* experiments. Furthermore, when explants and synovial cultures from healthy controls are cultured in the presence of exogenously added inflammatory components (e.g. oncostatin M, TNF-a, IL-1b, IL-6, IL-17A and F, IL-15, etc.), or alternatively, in the presence of synovial fluid from RA patients (which would contain inflammatory components endogenously), inflammatory and degradative pathway signaling can be observed. Therapeutics that would be efficacious in human RA *in vivo* would work in the above *in vitro* and *ex vivo* models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators.

[491] In these models, human synovial explants are collected from patients with RA, OA, or from healthy controls undergoing joint replacement or from post-mortem tissue collection, and processed using a modification of Wooley and Tetlow (Arthritis Res 2: 65-70; 2000) and van 't Hof et al (Rheumatology 39:1004-1008; 2000). Cultures of synovial fibroblasts, synovial macrophages and articular chondrocytes are also studied. Replicate samples are treated with one of the following: vehicle (PBS); recombinant human (rh) IL-17A; rhIL-17F; or rhIL-17A+rhIL-17F, and some samples contain various combinations of oncostatin M, TNF-a, IL-1b, IL-6, IL-17A, IL-17F, and IL-15. A separate set of samples are treated with activated human T cells, or synovial fluid from healthy controls or patients with RA or OA. In addition, all of these samples are treated with or without a soluble polypeptide of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158). After varying time of culture (from 1 h to several days), supernatants and cells are collected and analyzed for levels of inflammatory mediators and cartilage/bone/matrix biomarkers, including those listed above. In samples from patients with RA or OA, or in samples treated with RA/OA synovial fluid, activated T cells, rhIL-17A and/or rhIL-17F (either alone or in combination with other inflammatory cytokines), levels of inflammatory cytokines and chemokines and cartilage/bone/matrix degradative markers are elevated compared to untreated healthy control explants or in untreated cell cultures. The addition of a soluble polypeptide of the present invention markedly reduces the production of inflammatory and cartilage/bone/matrix degradative mediators, and thus, would expect to be efficacious in human RA and OA.

EXAMPLE 44

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Human Inflammatory Bowel Disease ("IBD") Samples via Mucosal Biopsy Cultures

[492] This model is designed to show that cultured intestinal tissue from patients with IBD produce higher levels of inflammatory mediators compared to tissue from healthy controls. This enhanced production of inflammatory mediators (including but not limited to IL-1b, IL-4, IL-5, IL-6,

IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, MCP-1, G- and GM-CSF, etc.) contributes to the symptoms and pathology associated with IBD such as Crohn's disease (CD) and ulcerative colitis (UC) by way of their effect(s) on activating inflammatory pathways and downstream effector cells. These pathways and components then lead to tissue and cell damage/destruction observed in vivo. Therefore, this model can simulate this enhanced inflammatory mediator aspect of IBD. Furthermore, when intestinal tissue from healthy controls or from human intestinal epithelial cell (IEC) lines is cultured in the presence of these inflammatory components, inflammatory pathway signaling can be observed, as well as evidence of tissue and cell damage.

[493] Therapeutics that would be efficacious in human IBD in vivo would work in the above ex vivo or IEC models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators.

[494] In this model, human intestinal tissue is collected from patients with IBD or from healthy controls undergoing intestinal biopsy, re-sectioning or from post-mortem tissue collection, and processed using a modification of Alexakis et al (Gut 53:85-90; 2004). Under aseptic conditions, samples are gently cleaned with copious amounts of PBS, followed by culturing of minced sections of tissue, in the presence of complete tissue culture media (plus antibiotics to prevent bacterial overgrowth). Samples from the same pool of minced tissue are treated with one of the following: vehicle (PBS); recombinant human (rh) IL-17A; rhIL-17F; or rhIL-17A+rhIL-17F. In addition, these are treated with or without a soluble polypeptide of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158). This experimental protocol is followed for studies with human IEC lines, with the exception that cells are passaged from existing stocks. After varying times in culture (from 1 h to several days), supernatants are collected and analyzed for levels of inflammatory mediators, including those listed above. In samples from patients with IBD or in samples treated with rhIL-17A and/or F, levels of inflammatory cytokines and chemokines are elevated compared to untreated healthy control tissue samples. The addition of a soluble polypeptide of the present invention markedly reduces the production of inflammatory mediators, and thus, would expect to be efficacious in human IBD.

[495] An additional arm of this study can include comparisons of the production of inflammatory mediators from tissue biopsies of IBD patients undergoing effective treatment, and those either not currently taking medications or considered non-responders to treatment.

EXAMPLE 45

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Human IBD Samples via Epithelial Barrier Function

[496] Maintenance of epithelial barrier integrity is a critical factor in the preservation of a healthy gastrointestinal tract. Experimental evidence suggests that leakiness of the epithelial barrier in the gut may contribute to the development of IBD. Immune cells located in the intestinal lamina propria generally interact with intestinal epithelial cells via cell to cell contact or production of soluble factors to maintain immune surveillance and contribute to epithelial barrier integrity. However, prolonged or dysregulated immune-mediated inflammation may contribute to defects in epithelial barrier cell integrity and function. The following study is designed to measure the direct effect(s) of T cell-derived IL-17A and/or IL-17F on epithelial barrier integrity.

[497] In this example, intestinal epithelial cell lines, like Caco-2 cells, are differentiated on semipermeable membranes and co-cultured on the basolateral side with either T cells or monocytes derived from biopsies from IBD patients or normal individuals. Epithlelial monolayer integrity is monitored over time using assessment of transepithelial electrical resistance or resistance of the monolayer to dye diffusion. Decreases in transepithial resistance of monolayers in co-cultures would suggest a disruption in the monolayer induced by the activity of the T cells or monocytes in the co-culture. Inhibitors of IL-17A and IL-17F such as the soluble polypeptides of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) could be used to determine the relative contribution of IL-17A and IL-17F to the disruption of the epithelial monolayer and test whether inhibitors of IL-17A and IL-17F would be effective in maintaining epithelial barrier integrity. Prevention of epithelial monolayer disruption induced by activated T cells by such molecules would suggest that the soluble IL-17RC and IL-17RC/IL-17RA polypeptides of the present invention may be effective for the therapeutic treatment of IBD in humans.

[498] Co-culture systems could also be generated using monolayers formed by primary epithelium from IBD patients to determine whether these cells are more sensitive to IL-17A and IL-17F compared to epithelial cells derived from healthy individuals. If so, these data would suggest that inhibiting IL-17A and IL-17F would be a suitable strategy for the therapeutic treatment of IBD.

EXAMPLE 46

Effects of IL-17A and IL-17F on Lamina PropPria T cells and Monocytes/Macrophages from Normal and Human IBD Samples

[499] Dysregulated or sustained immune-mediated inflammation may contribute to the symptoms and pathology associated with IBD by way of tissue damage or permanent skewing to inappropriate or prolonged immune responses. This model can determine the potential down-stream consequences of exposure of disease-associated T cells and monocytes to IL-17A and IL-17F which may be present in the immediate environmental cytokine mileu of the intestinal tissue.

[500] Therapeutics that would be efficacious in human IBD in vivo would work in the above ex vivo models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators (including but not limited to IL-1b, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, MCP-1, G- and GM-CSF, etc.).

[501] In this model, T cells and monocytes/macrophages are isolated from biopsy samples by carefully mincing biopsies with scissors in HBSS, treating with collagense and Dispase II and incubating for 1 hr at 37oC in a shaker. The cell suspension is filtered through nylon mesh to remove debris and cell clumps and washed multiple times in HBSS. T cells and macrophage/monocytes can be isolated using direct cell sorting or bead-depletion/enrichment protocols. Isolated cells are incubated in the presence of IL-17A and IL-17F. This induces the production of inflammatory mediators by T cells and monocytes/macrophages or results in skewing subsequent T cell responses to highly pro-inflammatory responses. Comparisons between the types of inflammatory mediators produced by cells from IBD patients and those from cells of normal individuals can be made and might suggest that T cells and monocyte/macrophages from IBD patients produce a more pro-inflammatory profile in the presence of IL-17A and IL-17F. The addition of a soluble polypeptide of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) to neutralize the production of downstream inflammatory mediators induced by IL-17A and IL-17F suggests that such soluble IL-17RC and IL-17RC/IL-17RA polypeptides may be efficacious in the therapeutic treatment of patients with IBD.

EXAMPLE 47

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Irritable Bowl Syndrome ("IBS"): CNS-Directed Pathogenesis

[502] A model focusing on primary CNS-directed pathogenesis of IBS which employs stress stimuli to induce symptoms characteristic of IBS. The neonatal psychosocial stress model

mimics some clinical features associated with IBS patients including visceral hyperalgesia, diarrhea and stress-sensitivity. Daily separation of the litter from their mothers for 180 minutes each day during postnatal days 4-18 will result in an alteration of maternal behaviour and significantly reduce times of the licking/grooming behaviour. The stress on the neonates results in permanent changes in the CNS resulting in altered stress-induced visceral and somatic pain sensitivity. Colonic motor function in response to stress is enhanced in these animals and preliminary data shows evidence of increased intestinal permeability (Mayer et al., 2002). Treatment with a soluble polypeptide of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) and subsequent analysis of colonic motor function, epithelial permeability and response to stress stimuli could determine efficacy in this animal model of IBS. Decreases in the incidence of symptoms following treatment with these inhibitors would suggest potential efficacy in the treatment of IBS.

EXAMPLE 48

Efficacy of the Soluble IL-17RC and IL-17RC/IL-17RA Polypeptides in Irritable Bowl Syndrome ("IBS"): Primary Gut-Directed Inducers of Stress

[503] This is a model focusing on primary gut-directed inducers of stress (ie. gut inflammation, infection or physical stress). Animal studies have indicated that low-grade inflammation or immune activation may be a basis for altered motility, and/or afferent and epithelial function of the gut (Mayer et al,. 2002). In this model, daily colon irritation is produced in neonatal animals (days 8-21) in the form of daily intracolonic injection of mustard oil. Mustard oil is a neural stimulant and has been shown to induce visceral hyperalgesia following intracolonic administration. This model mimics key features of the IBS including visceral hypersensitivity and alteration in bowel habits. Animals also present with diarrhea or constipation, a key feature of IBS patients (Mayer et al., 2002; Kimball et al., 2005). A soluble polypeptide of the present invention, such as a soluble IL-17RC polypeptide or a soluble IL-17RC/IL-17RA polypeptide (SEQ ID NO:158) could be delivered to determine changes in the development of symptoms associated with this model. Decreases in the incidence or magnitude of visceral hypersensitivity and altered gut motility following therapeutic treatment with our inhibitors would suggest a potential for these molecules to be efficacious in the treatment of IBS.

EXAMPLE 49

Designing a Scalable Protein Production Process for a Soluble IL-17A and IL-17F Antagonist

[504] In designing strategies focused on developing a scaleable protein production process for a soluble form of IL-17RC, many difficulties were encountered with identifying an expression system that allowed high level protein concentrations in the conditioned media. Western blot analysis demonstrated low levels of protein secretion with protein accumulating in the cell. In the discovery of the soluble polypeptides of the present invention, more than seventy different expression constructs were designed, generated, and tested for expression in either BHK cells, CHO cells, or HEK 293 cells. Several were tested in more than one host cell lines. Variations of tested soluble IL-17RC expression cassette included:

1) Alternative signal sequences such as: a) native; b) otPA; c) mouse immunoglobulin heavy chain variable region; d) human growth hormone; e) mouse IL17RA.

2) Two different naturally occurring splice variants (IL-17RCx1, SEQ ID NO:2; and IL-17RCx4, SEQ ID NO:166).

3) Addition of linker sequences between the IL-17RC extracellular domain (ECD) and the Fc portion, such as: a) no linker; b) a 9 amino acid linker based on GlyGlyGlySer; and c) a 20 amino acid linker based on GlyGlyGlySer.

4) His tagged monomeric forms.

5) Both amino- and carboxyl-terminal Fc fusion proteins.

6) Removal of N-linked carbohydrate attachment sites.

7) Gln for Asn amino acid substitutions.

8) Hybrid fusion proteins between IL17RA and IL17RC

[505] All of the soluble IL-17RC variant expression constructs were tested for protein expression by transient transfection in HEK 293 cells. Western blot analysis was used to detect protein secreted into the conditioned medium compared to protein retained in the cell by sampling cell lysates. Most of the constructs expressed protein secreted into the conditioned medium that was barely detectable by Western Blot. Additionally, the signal was greater from the cell lysate sample in comparison to the conditioned media sample indicating an inability for the protein to be efficiently secreted. Those expression constructs that resulted in the highest signals in the conditioned media were used to transfect stable CHO cell pools. Protein titers were measured from the stable CHO pools and where possible, purified protein was analyzed for IL-17A and IL-17F binding in a cell based competition binding assay. The following table shows protein expression results from the highest

expressing constructs in CHO cell stable pools. Where absolute protein concentration measurements were below the level of detection, the protein titer is indicated as < 0.5 mg/mL.

[506] IL-17RC and IL-17RC/RA protein expression constructs number designation, brief description of exons included, protein titer from stably transfeced CHO cell pools, and IL17A and IL17F binding ability. Not all the sequences of the variants included in Table 13 were included herewith.

Description	Protein Titer (mg/L)	Binding
x1 splice variant IL17RC exons 1-6, exons 8-16 (Variant 1210)	3.0	Ability to Block IL17A and IL17F
X4 splice variant IL17RC exons 1-16	< 0.5	Unable to obtain enough sample
IL17RC exons 1-6	< 0.5	Inactive
IL17RC exons 8-13	1.6	Inactive
IL17RC exons 7-16	< 0.5	Ability to Block IL17A and IL17F
IL17RA exons 1-10 IL17RC exons 8-16 (Variant 1407)	32.5	Ability to Block IL17A and IL17F
IL17RA exons 1-6 IL17RC exons 8-16 IL17RA exons 7-10	< 0.5	Inactive
IL17RA exons 1-3 IL17RC exons 4-16	< 0.5	Unable to obtain enough sample
IL17RA exons 1 IL17RC exons 2-16	< 0.5	Unable to obtain enough sample
IL17RA exons 1-6 IL17RC exons 8-16 (Variant 1454)	19	Ability to Block IL17A and IL17F

[507] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

PCT

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1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
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1-3-1	Name of depositary institution	ATCC American Type Culture Collection
1-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
1-3-3	Date of deposit	17 June 2003 (17.06.2003)
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CLAIMS

What is claimed is:

1. An isolated soluble polypeptide comprising at least one exon from IL-17RA (SEQ ID NO:21) and at least one exon from IL-17RC.

2. The isolated soluble polypeptide of claim 1, wherein the polypeptide sequence of IL-17RC is selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:166, SEQ ID NO:4, and SEQ ID NO:24.

3. The isolated soluble polypeptide of claim 1, wherein said soluble receptor binds to IL-17F.

4. The isolated soluble polypeptide of claim 3, wherein said soluble polypeptide further binds to IL-17A.

5. The isolated soluble polypeptide of claim 1, wherein said soluble polypeptide specifically binds to both IL-17F and IL-17A.

6. The isolated soluble polypeptide of claim 1, wherein said soluble polypeptide further comprises a polypeptide selected from the group consisting of: SEQ ID NO: 175 and SEQ ID NO: 180.

7. The isolated polypeptide of claim 1, wherein the polypeptide further comprises PEGylation.

8. An isolated soluble polypeptide comprising exons 8-16 of IL-17RC (amino acid residues 193-447 of SEQ ID NO:2), wherein said soluble polypeptide specifically binds to IL-17A and IL-17F.

9. The isolated soluble polypeptide of claim 8, wherein said polypeptide further comprises at least exon 1 of IL-17RA.

10. The isolated soluble polypeptide of claim 8, wherein said soluble polypeptide comprises exons 1-6 of IL-17RA.

11. The isolated soluble polypeptide of claim 8, wherein said polypeptide comprises the polypeptide depicted in Figure 1.

12. The isolated soluble polypeptide of claim 8, wherein said soluble polypeptide further comprises a polypeptide selected from the group consisting of: SEQ ID NO: 175 and SEQ ID NO: 180.

13. The isolated polypeptide of claim 8, wherein the polypeptide further comprises PEGylation.

14. An isolated soluble polypeptide comprising amino acid residues 1-458 of SEQ ID NO:158.

15. The isolated soluble polypeptide of claim 14, wherein the polypeptide comprises SEQ ID NO:158.

16. The isolated soluble polypeptide of claim 14, wherein the polypeptide consists of amino acid residues 1-458 of SEQ ID NO:158.

17. The isolated soluble polypeptide of claim 15, wherein the polypeptide consists of SEQ ID NO:158.

18. The isolated soluble polypeptide of claim 14, wherein the polypeptide further comprises PEGylation.

19. The isolated soluble polypeptide of claim 15, wherein the polypeptide further comprises PEGylation.

20. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: amino acid residues 193-276 of SEQ ID NO:2, amino acid residues 208-291 of SEQ ID NO:166, amino acid residues 277-370 of SEQ ID NO:2, amino acid residues 292-385 of SEQ ID NO:166, amino acid residues 371-447 of SEQ ID NO:2, and amino acid residues 386-462 of SEQ ID NO:166

21. The isolated polypeptide of claim 20, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 160, SEQ ID NO:162 and SEQ ID NO:164.

22. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:78, SEQ ID NO:82, SEQ ID NO:86, SEQ ID NO:90, SEQ ID NO:94, SEQ ID NO:98, SEQ ID NO:102, SEQ ID NO:106, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:118, SEQ ID NO:122, SEQ ID NO:140, and SEQ ID NO:152.

23. The isolated polypeptide of claim 22, wherein the polypeptide further comprises PEGylation.

24. A method of producing an antibody to a polypeptide comprising: inoculating an animal with a polypeptide selected from the group consisting of SEQ ID NO: 160, SEQ ID NO:162 and SEQ ID NO:164; and wherein the polypeptide elicits an immune response in the animal to produce the antibody; and isolating the antibody from the animal; and wherein the antibody specifically binds to an IL-17RC polypeptide; and reduces the activity of either IL-17A and/ or IL-17F.

25. The method according to claim 24, wherein the antibody produced by the method reduces the pro-inflammatory activity of either IL-17A and/ or IL-17F.

26. The method of claim 24, wherein the antibody produced by the method neutralizes the interaction of either IL-17A and/ or IL-17F with IL-17RC or IL-17RA.

27. The method of claim 26, wherein the neutralization by the antibody is measured by showing neutralization of either IL-17A and/ or IL-17F in an *in vitro* a cell-based neutralization assay.

28. The method of claim 24, wherein the antibody produced by the method reduces the pro-inflammatory activity of both IL-17A and IL-17F.

29. The method of claim 24, wherein the antibody produced by the method neutralizes the interaction of both IL-17A and IL-17F with IL-17RC.

30. The method of claim 26, wherein the neutralization by the antibody is measured by showing neutralization of both IL-17A and IL-17F in an *in vitro* a cell-based neutralization assay.

31. A method for reducing or inhibiting either IL-17A-induced or IL-17F-induced inflammation comprising administering to a mammal with inflammation an amount of a soluble polypeptide according to any of claims 1, 8, 14 or 15 sufficient to reduce inflammation.

32. A method of reducing IL-17A-induced and IL-17F-induced -induced inflammation comprising administering to a mammal with inflammation an amount of a soluble polypeptide according to any of claims 1, 8, 14 or 15 sufficient to reduce inflammation.

33. A method of treating a mammal afflicted with an inflammatory disease in which IL-17A or IL-17F plays a role, comprising: a) administering an antagonist of IL-17A or IL-17F to the mammal such that the inflammation is reduced, wherein the antagonist comprises a soluble polypeptide according to claim 1, and wherein the inflammatory activity of either IL-17A or IL-17F is reduced.

34. The method of claim 33, wherein the disease is asthma.

35. The method of claim 33, wherein the disease is a chronic inflammatory disease.

36. The method of claim 35, wherein the disease is a chronic inflammatory disease comprising inflammatory bowel disease, ulcerative colitis, Crohn's disease, arthritis, atopic dermatitis, or psoriasis.

37. The method of claim 33, wherein the disease is IBS.

38. The method of claim 33, wherein the disease is an acute inflammatory disease.

39. The method of claim 38, wherein the disease is an acute inflammatory disease comprising endotoxemia, septicemia, toxic shock syndrome or infectious disease.

40. A method of treating a mammal afflicted with an inflammatory disease in which IL-17A and IL-17F plays a role, comprising: a) administering an antagonist of IL-17A and IL-17F to the mammal such that the inflammation is reduced, wherein the antagonist comprises a soluble polypeptide according to claim 1, and wherein the inflammatory activity of either IL-17A and IL-17F is reduced.

41. The method of claim 40, wherein the disease is asthma.

42. The method of claim 40, wherein the disease is a chronic inflammatory disease.

43. The method of claim 42, wherein the disease is a chronic inflammatory disease comprising inflammatory bowel disease, ulcerative colitis, Crohn's disease, arthritis, atopic dermatitis, or psoriasis.

44. The method of claim 40, wherein the disease is IBS.

45. The method of claim 40, wherein the disease is an acute inflammatory disease.

46. The method of claim 45, wherein the disease is an acute inflammatory disease comprising endotoxemia, septicemia, toxic shock syndrome or infectious disease.

47. The method of claim 33, wherein the disease is multiple sclerosis.

48. The method of claim 40, wherein the disease is multiple sclerosis.

49. The method of claim 33, wherein the disease is rheumatoid arthritis.

50. The method of claim 40, wherein the disease is rheumatoid arthritis.

51. The method of claim 33, wherein the disease is osteoarthritis.

52. The method of claim 40, wherein the disease is osteoarthritis.