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(54) Title: METHODS FOR MODULATING ACTIVITY OF IL-18 FOR TREATMENT OF CD4+ T CELL MEDIATED AUTOIMMUNITY AND IMMUNOPATHOLOGY

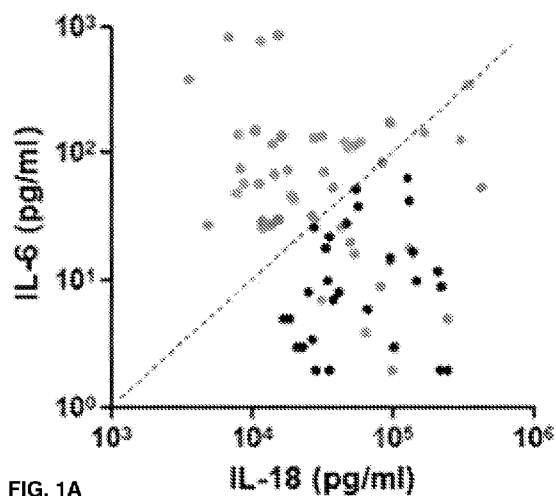


FIG. 1A

(57) Abstract: Compositions and methods for modulating IL- 18 activity for amelioration of aberrant inflammatory symptoms associated with increased autoreactivity of CD4+ T cells are disclosed.

**Methods for Modulating Activity of IL-18 for Treatment of
CD4+ T cell Mediated Autoimmunity and Immunopathology**

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Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Patent Application No.
10 63/512,789, filed July 10, 2023, the entire contents being incorporated herein as though set forth
in full.

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15 **Incorporation-by-Reference of Material Submitted in Electronic Form**

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Field

This invention relates to the fields of immunological and inflammatory disorders,
20 particularly, disorders associated with, or caused by, auto-reactive or otherwise pathogenic T
cells (CD4+, CD4-, CD8+, regulatory T cells, natural killer (NK) cells), and antigen presenting
cells (APCs) and methods for treatment thereof with agents which enhance or augment the
immune-protective activity of the cytokine Interleukin 18 (IL-18).

Background

25 Several publications and patent documents are cited throughout the specification in order
to describe the state of the art to which this invention pertains. Each of these citations is
incorporated herein by reference as though set forth in full.

IL-18 is an IL-1 family cytokine with both pro-inflammatory and immune-protective

functions. IL-18 is thought to drive and/or exacerbate the “autoinflammatory” category of immune dysregulation disorders associated with extremely elevated levels of IL-18 in circulation. These include Systemic Juvenile Idiopathic Arthritis (sJIA)^{1,2}, Adult-Onset Still's Disease (AOSD)³, Macrophage Activation Syndrome (MAS, often a complication of sJIA or AOSD), and several ultra-rare autoinflammatory Inborn Errors of Immunity (IEI) including diseases associated with gain-of-function mutations in NLRC4^{1,4} or PSTPIP1⁵, deficiency of XIAP⁶, or specific c-terminal mutations in CDC42^{7,8}.

In contrast with autoinflammatory diseases, there are several diseases in which symptoms are caused by pathogenic CD4 T-cells. Diseases wherein CD4+ T cells are likely pathogenic include those with a strong association with specific Major Histocompatibility Complex (MHC) class II alleles (MHCII), i.e. those encoding HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR. There have been extensive studies to understand the association between variations of human leukocyte antigens (HLAs) and various diseases⁹. Clearly a need exists for new agents and treatment modalities to prevent and/or alleviate symptoms caused by pathogenic CD4+ T cells.

Autoimmune diseases with MHCII associations (and thereby likely to be caused by CD4+ T-cells) include but are not limited to multiple sclerosis (MS), rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1D), Autoimmune thyroiditis (both Graves and Hashimoto's), autoimmune neutropenia, autoimmune hemolytic anemia, immune thrombocytopenic purpura, cold agglutinin disease, Pemphigus Vulgaris, and Myasthenia Gravis. In addition to defects in HLAs, these autoimmune diseases are also associated with autoreactive or pathogenic antigen specific CD4+ T cells⁹. Similar inflammatory diseases with MHCII associations where the pathogenic T-cell reactivity may not be to self, include, but are not limited to, ulcerative colitis (UC) and celiac disease (CeD). Pathogenic CD4s are also seen in allergic disorders like atopic dermatitis, allergic rhinosinusitis, asthma, and eosinophilic gastritis/esophagitis as well as in graft-versus-host-diseases (GvHD).

There is a growing amount of literature suggesting the existence of suppressive, regulatory CD8+ T cells that have the capacity to inhibit the function and or proliferation of autoreactive CD4+ T cells to reduce autoimmunity¹². Recently published data from *Li et al* have described a specific subset of human suppressive CD8s as KIR+CD8+ cells that are the human equivalent to Ly49+ CD8+ cells in mice¹³. Our recent experiments in murine models suggest

that, in conditions of pathologic CD4 T-cell activation, IL-18 may specifically enhance the number and/or function of suppressive CD8 T-cells and/or regulatory CD4 T-cells to limit/prevent immunopathology mediated by pathogenic CD4 T-cells. Thus, new agents and treatment modalities that mimic the beneficial effects of IL-18 may prevent and/or alleviate symptoms caused by pathogenic CD4+ T cells.

It is an object of the present inventors to provide new treatment modalities to alleviate symptoms caused by pathogenic immune cell signaling.

Summary

New agents and treatment modalities that utilize the beneficial effects of IL-18 signaling for inhibiting onset of, and/or alleviating symptoms caused fully or partially by pathogenic CD4+ T cells are disclosed. The data presented herein demonstrate that, in a murine model of CD4 T-cell driven autoimmunity, excess IL-18 acts on CD8 T cells to prevent and/or ameliorate symptoms of autoimmunity. Our recent work has established that, in systems where pathogenic autoreactive CD4 T-cells are opposed by suppressor CD8 T-cells, IL-18 may specifically promote the function of the latter to substantially prevent or improve symptoms of autoimmunity. We thus hypothesized that IL-18, and agonists thereof, can be utilized to augment the number and/or function of suppressor cytotoxic cells to prevent autoimmunity by direct or indirect inhibition of pathogenic, autoreactive CD4 T cells.

In certain aspects, a method for inhibiting pathogenic immune cell activities, (e.g., by lymphocytes like CD4+ T-cells, CD8+ T-cells, gamma/delta T-cells, Natural Killer T-cells, and/or cells providing stimulation to these lymphocytes including various antigen presenting cells) and ameliorating disease activity in autoimmune, allergic, or post-transplant disorders in a subject in need thereof is provided. An exemplary method comprises administering to the subject an effective amount of an agent that mimics beneficial immune protective effects of Interleukin-18 (IL-18) to promote the differentiation, expansion, and, or function of therapeutic suppressive immune cells (e.g., CD8⁺ T-cells, CD4+ T-regulatory cells, NK cells, or other IL-18 responsive suppressive immune cell), thereby inhibiting immunopathology in said subject; or administering to the subject an effective amount of a cellular therapeutic comprising cytotoxic cells cultured or conditioned in an agent that mimics beneficial immune protective effects of IL-

18, thereby inhibiting immunopathology resulting from pathogenic cells in said subject; and monitoring said subject for improvement in symptoms mediated by pathogenic cells.

In certain embodiments of the method, the T-cells causing immunopathology are autoreactive. Disorders to be treated include, without limitation, multiple sclerosis, rheumatoid arthritis, myasthenia gravis, Autoimmune encephalitis, Addison's disease, celiac disease, type 1 diabetes mellitus, autoimmune thyroiditis, inflammatory bowel disease (IBD, ulcerative colitis, Crohn's Disease, Sjogren syndrome, systemic lupus erythematosus, Grave's disease, Crohn's disease, Waldenstrom's macroglobulinemia, hyperviscosity syndrome, monoclonal gammopathy of undetermined origin, POEMS syndrome, myeloma, and macroglobulinemia.

In other embodiments, the disorder is an allergic disorder selected from atopic dermatitis, allergic rhinosinusitis, allergic asthma, and eosinophilic enteritis. In cases where the disorder is a post-transplant disorder, the disorder can be graft versus host disease (GvHD).

The agent used in the method can increase bioactivity of free IL-18 in said subject. In other aspects, the agent reduces binding of IL-18 binding protein (IL-18BP). In some instances, the agent increases bioactivity of free IL-18 and while simultaneously reducing IL-18BP binding. In other embodiments, the agent is a functional IL-18 mimetic, fusion protein, antibody, peptibody, mimic or a decoy resistant IL-18 (DR-18) which, upon delivery to the patient, augments the immunoprotective functions of IL-18. The agent can be administered via any route including a route selected from systemic, intravenous, subcutaneous, topical, aerosolized, or oral administration. In yet another aspect, the differentiation, expansion, and, or function of suppressive CD8+ T cells or the protective IL-18 functions induced by said agent in the patient is monitored.

The IL-18 (DR-18) molecule can comprise an amino acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with at least one of SEQ ID NOs: 2-63. In certain embodiments, the DR-18 comprises one or more mutations at positions Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110, N111, M113, V153, and N155 relative to the wild-type IL-18 amino acid sequence set forth in SEQ ID NO:108. In other embodiments, DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 20, 7, 17-19, 21, 22, 55-57, 62 and 63.

In methods of administration of DR-18 molecule or variants thereof, the amount of the DR-18 administered is from 15 µg/kg to 3000 µg/kg. The agent, agonist or antagonist described herein can be administered via a route selected from systemic, intravenous, or subcutaneous administration. In certain aspects of the method, differentiation, expansion, and, or function of suppressive CD8+ T cells induced by said agent is monitored.

In other embodiments, wherein signaling from said APC engages MHC class II molecules which interact with T cell receptors, said APC being selected from dendritic cells, macrophages, B cells, thymic epithelial cells, vascular endothelial cells, and follicular dendritic cells.

In yet another aspect of the methods provided above, immune protective effects of free IL-18 can include one or more of i) binding of IL-18 to the IL-18R and activation of the IL-18R; ii) regulation of innate and acquired immune responses; iii) induction of one or more T-lymphocyte helper (Th1) responses; iv) enhanced cell-mediated cytotoxicity; v) IFN-γ induction; vi) enhanced production of GM-CSF and IL-2; vii) potentiation of anti-CD3 induced T-cell proliferation; viii) increased Fas-mediated killing by natural killer cells (NK cells) and CD4+ Th1 cells; ix) increased apoptotic death via activation of the Fas-FasL pathway signaling; x) up-regulation of FasL expression; xi) induction of T-lymphocyte helper cell type 2 responses (Th2) in T-cells and NK cells; xii) stimulation of basophils and mast cells to produce Th2 cytokines and histamine; xiii) inhibition of IgE production; and xiv) activation of T cell signaling pathways by APC cells.

In certain embodiments, the antagonist is selected from an inhibitory nucleic acid, an inhibitory antibody, a peptide mimetic, a small molecule, or an IL-18 decoy-to-the-decoy (D2D). In other embodiments, the IL-18 D2D comprises an amino acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with at least one of SEQ ID NOs: 64-97. In certain instances, the DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 64-97.

The methods described herein can further comprise administration of one or more anti-inflammatory agents selected from analgesic agents, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs). Other agents to be administered include, for example, one or more antiviral, antibiotic, analgesic, corticosteroid, antagonists of

inflammatory cytokine, a non-steroidal anti-inflammatory agent, pentoxifylline, thalidomide, azathioprine, cyclophosphamide, cyclosporine, hydroxychloroquine sulfate, methotrexate, leflunomide, minocycline, penicillamine, sulfasalazine, indomethacin; celecoxib; rituxin, rofecoxib; ketorolac; nambumetone; piroxicam; naproxen; oxaprozin; sulindac; ketoprofen; diclofenac; and gold compounds such as oral gold, gold sodium thiomalate, and aurothioglucose.

In certain aspects of the method, the culturing or conditions produce Suppressive CD122+CD8+ T-cells and/or KIR+CD8+ T-cells. In other aspects, the methods employ donor cells haplotype-matched to the subject to be treated. In other aspect, cells are autologous and harvested from the subject to be treated.

Use of a composition comprising an agonist of interleukin 18 (IL-18) or IL-18 receptor (IL-18R), in a method of treating a subject, is also provided, wherein the subject is a human subject having a disease or disorder selected from the group consisting of: autoimmune disease or disorder, allergic disease or disorder, post-transplant disease or disorder, and an inflammatory disease caused by pathogenic CD4+ T cells; and the method treats the subject for the disease or disorder. In certain embodiments, the disease or disorder is multiple sclerosis. The agonist to be administered can be a decoy resistant IL-18 (DR-18) and can comprises an amino acid sequence selected from SEQ ID NOs: 20, 7, 17-19, 21, 22, 55-57, 62 and 63.

Also disclosed is use of a composition comprising an antagonist of interleukin 18 binding protein (IL-18BP), in a method of treating a subject, wherein the subject is a human subject having a disease or disorder selected from the group consisting of: autoimmune disease or disorder, allergic disease or disorder, post-transplant disease or disorder, and an inflammatory disease caused by pathogenic CD4+ T cells; and the method treats the subject for the disease or disorder. In certain approaches, the disease or disorder is multiple sclerosis, and the antagonist is an interleukin 18 decoy-to-the-decoy (IL-18 D2D). In other approaches, the antagonist is an IL-18BP inhibitory antibody that specifically binds IL-18BP.40. The use also includes the antagonist is an IL-18BP inhibitory nucleic acid that prevents translation and/or expression of a nucleic acid encoding IL-18BP.

Brief Description of the Drawings

Figures 1A – 1F: Excess IL-18 protects from Experimental Autoimmune Encephalomyelitis (EAE). Fig. 1A [Prior Art]: Extracted from Shimizu *et al.*,¹⁷. Two systemic juvenile idiopathic

arthritis (s-JIA) subsets: IL-6-dominant subset (red circles), IL-18-dominant subset (black circles). Patients complicated with MAS (green circles). Patients with higher IL-18/IL-6 ratio had significantly less inflammatory arthritis. Fig. 1B: Excess IL-18 is protective against clinical onset features of (EAE). Mean clinical EAE scores of *Il18bp^{-/-}* and WT mice immunized with “myelin oligodendrocyte glycoprotein (MOG35–55) in CFA to induce EAE (n=9). Fig. 1C: Mean clinical scores of *Il18tg* and WT mice (n=10). Fig. 1D recombinant murine mature IL-18 (1ug/injection) was administered IP into WT mice thrice on alternating days during induction phase (Days 0, 2, 4) or effector phase (Days 9, 11, 13) (n=8/group). Fig. 1E: Recombinant murine Decoy-Resistant IL-18 (DR-18, *Zhou et al.*¹⁴, 8ug/dose, or PBS was administered every 3 subcutaneously. Fig. 1F: IL-18 receptor blocking antibody (clone 9E6, 500ug/injection) was given IP at the indicated timepoints relative to EAE induction. ****p <0.0001, (Figs. 1A, 1B, 1C, 1E) repeated measures ANOVA. Fig. 1D Sidak pairwise comparison of indicated groups following two-way ANOVA.

Figures 2A -2C: Excess IL-18 promotes CD8, relative to CD4, T-cell activation in mice with Experimental Autoimmune Encephalomyelitis (EAE). Fig. 2A: Representative flow cytometry plots, and % CD8+ T-cells, of spinal cord cells obtained at day 21-25 post induction of EAE as in Fig. 1. Pre-gated on live T-cells. Unpaired T-test. Fig. 2B: Total cells/spinal cord from WT mice treated with DR-18 as in Fig. 1 same condition as in Fig. 1E. Fig. 2C: EAE was induced in mice of the indicated genotypes. 2D2 indicates mice bearing the 2D2 MOG-specific T-cell receptor transgene. *p <0.05, Sidak pairwise comparison of indicated groups following two-way ANOVA.

Figures 3A-3D: IL-18 sensing by CD8 T-cells is required for protection from Experimental Autoimmune Encephalomyelitis (EAE). Fig. 3A: EAE was induced as in Fig. 1 in mice of the indicated genotypes (*Il18r1^{ΔT}*=*CD4Cre^{pos}*; *Il18r1^{fllox/fllox}*. “*Il18tg* ctrls” in these experiments are *CD4Cre^{Neg}*; *Il18r1^{fllox/fllox}*; *Il18tg* littermate controls, where the *Il18tg* derives from Hoshino et al.¹⁸ Repeated measures ANOVA of non-WT groups. Fig. 3B: Proportion of spinal cord immune cells that were CD8+ T-cells day 21-25 post EAE induction. Unpaired t-test of indicated genotypes. Fig. 3C: EAE was induced as in Fig. 1 in mice of the indicated genotypes (*Il18r1^{ΔFoxP3}*=*FoxP3Cre*; *Il18r1^{fllox/fllox}*. *Il18tg* in these experiments derive from Cre-negative *Il18r1^{fllox/fllox}* littermate controls). Repeated Measures 2-way ANOVA with Sidak post-test of WT

vs Il18tg (top) and WT vs Il18tg;Il18r1^{ΔFoxP3} (bottom). Fig. 3D: EAE was induced as in Fig. 1 in mice of the indicated genotypes (Il18r1^{ΔCd8}=ERT2c8iCre;Il18r1^{flox/flox}), with ERT2c8iCre generated in as described in Andrews et al.¹⁹. All mice were administered tamoxifen 1mg IP on days 4 and 6 post-immunization. Efficient deletion of IL-18R on T-cells, Tregs, or CD8 T-cells was confirmed by flow cytometry.

Figures 4A-4B: Excess IL-18 alters CNS CD8 T-cell phenotype in EAE: EAE was induced in mice receiving DR-18 or PBS as in Fig. 1E. Fig. 4A: Activation state (CD44+) and representative flow plots of Day 21-25 spinal cord CD8 T-cells stained for Ly49 and (intracellular) Helios directly ex vivo. Fig. 4B: Quantitation of average (n=3-4 per group) Ly49 and Helios expression in CD8 T-cells from quadrants as in Fig. 4A.

Figures 5A-5C: IL-18 and autoreactive CD4 T-cells may augment/expand regulatory CD8 T-cells in Experimental Autoimmune Encephalomyelitis (EAE). Fig. 5A: Experimental design for Fig. 5B. EAE was induced in WT mice and splenocytes harvested on day 10 post-immunization. Splenocytes were cultured in media containing IL-2 alone or IL-2 + IL-18 for 3 days before FACS purification and injection of 5e6 cultured CD8 T-cells. Fig. 5C: 2D2-transgene expressing CD4 T-cells were FACS purified and transferred in the indicated numbers into Il18bp^{-/-} mice at the time of EAE induction. Repeated measures ANOVA of indicated groups, p>0.01.

Detailed Description

Interleukin-18 (IL-18) is an inflammatory cytokine that has long been associated with the induction of Interferon-gamma. Notably, it has a highly abundant, high-affinity natural antagonist called IL-18 binding protein (IL-18BP). IL-18 levels are modestly elevated in the serum of patients with a wide variety of inflammatory diseases from cancer to lupus, but as noted above, extremely high levels are observed in Systemic Juvenile Idiopathic Arthritis (SJIA) and several monogenic mimics of SJIA. One notable observation in SJIA is that the patients with more IL-18 tend to have more systemic inflammation, but paradoxically LESS inflammatory arthritis. (Fig. 1A). While the systemic inflammation appears to be caused more by innate immune hyperactivation, chronic arthritis is a canonically autoimmune finding associated with diseases like rheumatoid arthritis mediated by autoreactive T-cells and autoantibodies. We follow several patients whose genetic disease results in high levels of serum IL-18, and

detectable free IL-18 (unbound by IL-18BP) over their whole lives. Despite chronic inflammation, to date none of these patients has developed autoimmune manifestations. Cancer patients often have extremely high IL-18BP production. This has led to the development of a “decoy-resistant” version of IL-18 (DR-18) that is now in clinical development by Simcha Therapeutics as a cancer immunotherapy (DR-18¹⁴,) and described in clinical trial NCT04787042 titled “Phase 1a and Phase 2 Study for Safety, Preliminary Efficacy, PK and PD of ST-067” (further info available at [clinicaltrials\(dot\)gov/study/NCT04787042](https://clinicaltrials.gov/study/NCT04787042))).

IL-18 activity can be augmented in many ways. In vivo, excess IL-18 can be provided via administration of exogenous IL-18 or IL-18 analogues (like DR-18), via use of transgenic expression as in mice engineered to express IL-18 (*Il18tg*) or Chimeric Antigen Receptor (CAR) T-cells that express IL-18 (CART19-IL18)²⁰, The effectiveness of exogenous recombinant human IL-18 (rhIL-18) has been found to be limited by endogenous production of human IL-18BP. Therefore, in some instances, a DR-18 may be used. IL-18 signaling can be increased by inhibiting the production and/or activity of IL-18BP, for example via use of mice deficient in IL-18BP (*Il18bp*^{-/-}), administration of an IL-18BP antagonist, or the like. In these systems, for example via use of mice deficient in IL-18BP (*Il18bp*^{-/-}). Excess or unopposed IL-18 has been shown to exacerbate various models of systemic inflammation^{1, 15, 21}. CART19-IL18 and DR-18 has been shown to alleviate murine models of cancer by activating T- and NK-cell anti-tumor immune responses¹⁴. Notably, these are NOT models testing the effects of IL-18 on autoimmunity.

It is unclear how excess IL-18 affects antigen specific autoimmunity. A host of correlative work has associated elevated serum IL-18 levels with autoimmune disease and speculated at a role in promoting or exacerbating disease²²⁻²⁴. The experimental autoimmune encephalitis (EAE) murine model is an ideal system to interrogate the role of IL-18 on antigen-specific autoimmunity. EAE is caused by the expansion and Central Nervous System infiltration of T-cells reactive to a peptide present in myelin. Like nearly all activated T-cells, these autoreactive T-cells highly express the IL-18 receptor. Past research on the role of IL-18 in this model has had unclear results but did not focus on IL-18 excess. It is also clear in this model that a certain population of CD8 T-cells is actually protective against the autoimmune manifestations^{12, 25}.

Despite their excess IL-18 activity, we have found that EAE in *Il18tg* or *Il18bp^{-/-}* mice is far less severe than that observed in WT mice (Figures 1B, C). Provision of exogenous IL-18 is also partially protective against EAE in WT mice, particularly if given early in the course of disease possibly due to lower levels of IL-18BP early in disease (Figure 1D). This improvement in disease correlates with a significant increase in the proportion of activated CD8 T-cells in the CNS of *Il18bp^{-/-}* mice (Figure 2). By contrast, we very recently found that unopposed IL-18 became deleterious when baseline proportion of autoreactive CD4 T-cells relative to CD8 T-cells was altered (Figure 3). This was achieved by breeding mice with transgenic expression of a T-cell receptor specific for the MOG peptide (2D2 mice) with *Il18bp^{-/-}* mice. In 2D2 mice, nearly all of the T-cells are CD4 positive and recognize MOG. *Il18bp^{-/-}*_2D2 mice become sick incredibly quickly, more quickly than 2D2 mice with normal expression of *Il18bp*. In this case, these data reveal that unopposed IL-18 can actually promote T-cell mediated autoimmunity.

Thus, the present disclosure provides for the use of IL-18, functional mimetics thereof including DR-18, IL-18 receptor agonists, IL-18BP antagonists and IL-18-treated cellular therapies, to promote the differentiation of cells capable of restricting autoreactive CD4 T-cells, thereby providing therapeutic benefit to the subject. This could be both as an adjunct used in vitro in the development of cellular therapies, or in vivo in affected or at-risk patients. T-cells capable of suppressing pathologic CD4 T-cell responses have been observed not just in autoimmunity, but in graft-versus-host disease (GvHD). Thus, IL-18 can be used to advantage in promoting the expansion and activity of protective T-cells in CD4 T-cell mediated GvHD (Zheng J. et al., Human CD8+ regulatory T cells inhibit GVHD and preserve general immunity in humanized mice. *Sci Transl Med.* 2013 Jan 16;5(168):168ra9; Agle K. et al., Bim regulates the survival and suppressive capability of CD8+ FOXP3+ regulatory T cells during murine GVHD. *Blood.* 2018 Jul 26;132(4):435-447. doi: 10.1182/blood-2017-09-807156. Epub 2018 May 16; Dai Z. et al., Natural CD8+CD122+ T cells are more potent in suppression of allograft rejection than CD4+CD25+ regulatory T cells. *Am J Transplant.* 2014 Jan;14(1):39-48. doi: 10.1111/ajt.12515. Epub 2013 Nov 12).

Definitions:

The phrase “Myelin-associated autoimmune diseases” refers to central nervous system (CNS) demyelinating autoimmune diseases are autoimmune diseases which primarily affect

the central nervous system. Examples include, without limitation, diffuse cerebral sclerosis of Schilder, acute disseminated encephalomyelitis, acute hemorrhagic leukoencephalitis, multiple sclerosis, transverse myelitis and neuromyelitis optica.

“Experimental autoimmune encephalomyelitis (EAE)”, another myelin associated autoimmune disease is a widely used animal model that is characterized by an inflammatory attack against myelin components of the CNS. EAE is primarily mediated by CD4 T cells, which are directed against defined epitopes of CNS myelin. These self-reactive T cells are normal constituents of the peripheral T cell repertoire and can be activated in vivo by subcutaneous immunization with myelin antigens. The central role of CD4 T cells in EAE is demonstrated by their ability to transfer disease when injected into unchallenged hosts.

As used herein, “interleukin-18 (IL-18)”, also known as “interferon-gamma inducing factor” is a cytokine, which is produced by a variety of myeloid cells, activated macrophages (including Kupffer cells), and barrier epithelial cells of the intestine, skin, and lung. IL-18 binds to IL-18 receptor alpha (IL-18R α) and IL-18 receptor beta (IL-18R β) to form the IL-18 receptor complex, which formation and induces IL-18 signaling. References herein to “IL-18R” will refer to the IL-18 receptor generally either as an individual component thereof (e.g., as in IL-18R α , such as in the context of IL-18 binding to IL-18R α) or collectively as the receptor complex (e.g., as in formation of the complex through binding between IL-18, IL-18R α , and IL-18R β to form the signaling complex), which will be readily understood by the relevant skilled artisan. Recent murine evidence suggests IL-18 signaling in some instances may be mediated in part through a Na-Cl symporter (NCC, encoded by Slc12a3)(26-28), and the scope of this disclosure is not limited to the effects of IL-18 mediated solely through IL-18R α , IL-18R β , and/or NCC. IL-18 signaling is typically dependent on the adaptor MyD88 via canonical NF- κ B and MAPK pathways. Defects (e.g. knock-out) of the IL-18 receptor (IL-18R α and/or IL-18R β) or IL-18 lead to impaired natural killer (NK) cells activity and T-cell responses. Apart from its physiological role, excess IL-18 may also induce severe inflammatory disorders. For the purpose of early diagnosis of such disorders it therefore may be useful to quantify the levels of free IL-18 in body fluids of a subject, expected to have such a disorder. For example, recombinant IL-18 is described in US Patent Application 16/123,063 to Aaron Ring, which is incorporated herein by reference.

As used herein, the term “activity” refers to a biological activity.

As used herein, the term “pharmacological activity” refers to the inherent physical properties of a peptide, polypeptide or small molecule. These properties include but are not limited to half-life, solubility, and stability and other pharmacokinetic properties.

The terms “high,” “higher,” “increases,” “elevates,” or “elevation” refer to increases above basal levels, e.g., as compared to a control or reference level. The terms “low,” “lower,” “reduces,” or “reduction” refer to decreases below basal levels, e.g., as compared to a control or reference level.

The term “modulate” as used herein refers to the ability of a compound to change an activity in some measurable way as compared to an appropriate control. As a result of the presence of compounds in the assays, activities can increase or decrease as compared to controls in the absence of these compounds. In some instances, an increase in activity is at least 25%, at least 50%, at least 100% compared to the level of activity in the absence of the compound. Similarly, a decrease in activity is, in some instances, at least 25%, at least 50%, at least 100% compared to the level of activity in the absence of the compound. A compound that increases a known activity is an “agonist”. One that decreases, or prevents, a known activity is an “antagonist”.

The term “inhibit” means to reduce or decrease in activity or expression. This can be a complete inhibition or activity or expression, or a partial inhibition. Inhibition can be compared to a control or to a standard level. Inhibition can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%.

The term “in need of treatment” as used herein refers to a judgment made by a caregiver (e.g. physician, nurse, nurse practitioner, or individual in the case of humans; veterinarian in the case of animals, including non-human mammals) that a subject requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a care giver's expertise, but that includes the knowledge that the subject is ill, or will be ill, as the result of a condition that is treatable by the disclosed compounds.

The phrase “acceptable carrier” refers to a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the selected compound

without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Other compounds will be administered according to standard procedures used by those skilled in the art.

As used herein, “subject” includes vertebrates, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent). The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term “patient” includes human and veterinary subjects.

By “treatment” and “treating” is meant the medical management of a subject with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder. It is understood that treatment, while intended to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder, need not actually result in the cure, ameliorization, stabilization or prevention. The effects of treatment can be measured or assessed as described herein and as known in the art as is suitable for the disease, pathological condition, or disorder involved. Such measurements and assessments can be made in qualitative and/or quantitative terms. Thus, for example, characteristics or features of a disease, pathological condition, or disorder and/or symptoms of a disease, pathological condition, or disorder can be reduced to any effect or to any amount.

A cell can be *in vitro*. Alternatively, a cell can be *in vivo* and can be found in a subject. A “cell” can be a cell from any organism including, but not limited to, a bacterium, a yeast, a mammal, etc. A cell can be a eukaryotic cell or prokaryotic cell. In some instances, a cell is a human cell. In some instances, a cell may be obtained from a subject, treated, cultured, and/or

modified in some way, and then administered to the same subject in an autologous manner. In some instances, a cell may be obtained from a donor, treated, cultured, and/or modified in some way, and then administered to a different subject in an allogeneic manner. Accordingly, cell therapies may be “autologous” or “allogeneic”, as desired.

5 By the term “effective amount” of a compound as provided herein is meant a nontoxic but sufficient amount of the compound to provide the desired result.

By “pharmaceutically acceptable” is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with
10 any of the other components of the pharmaceutical composition in which it is contained.

An “antagonist” (also sometimes referred to herein as an “inhibitor”) as defined herein, is a molecule that decreases, or prevents, a known activity. An antagonist may directly inhibit a signaling pathway, e.g., by partially or completely blocks blocking the binding of two cognates or members of a specific binding pairs, thereby inhibiting the downstream biological effects of
15 the cognates or binding pair interaction. For example, an antagonist may block the binding of a ligand to its receptor, which in turn reduces and/or prevents intracellular signaling via activating that receptor, which in turn reduces or prevents the downstream biological effects of activating that receptor, such as but not limited to, cell activation, proliferation, differentiation, cytokine release, up-regulation of genes, cell-surface expression of proteins, and the like. Activating or
20 activation of a receptor is defined herein as the engagement of one or more intracellular signaling path way(s) and the transduction of intracellular signaling (i.e., signal transduction) in response to a molecule binding to a membrane-bound receptor, such as but not limited to, a receptor:ligand interaction.

In some instances, an antagonist may decrease or prevent the activity of an inhibitory
25 molecule, such as a ligand binding protein that binds to and inhibits the function of a ligand (also sometimes referred to as a “decoy” or “decoy receptor”). In such instances, the antagonist may inhibit the inhibitor, thereby increasing signal transduction associated with the ligand. For example, useful antagonists include IL-18BP antagonists, such as an anti-IL-18BP antibody or a IL-18 mimic that preferentially binds IL-18BP. Useful IL-18BP antagonists bind IL-18BP and
30 prevent it from binding to and inhibiting IL-18 (i.e., preventing IL-18BP inhibition of IL-18). An antagonist may also be a molecule that decreases or prevents the expression of a target, thereby

decreasing or preventing activity associated with the target, e.g., any signal transduction or inhibitory function associated with the target. For example, an antagonist may be a small molecule, such as e.g., an interfering nucleic acid (e.g., a small interfering RNA, a short-hairpin RNA, an antisense DNA or RNA, etc.) that decreases or prevents the expression of the target of the interfering nucleic acid. In some instances, an antagonist that decreases or prevents the expression of a ligand binding protein may be employed, thereby inhibiting the inhibitory function of the ligand binding protein. For example, an antagonist that decreases or prevents the expression of IL-18BP may be employed, thereby inhibiting IL-18BP mediated inhibition of IL-18 signaling.

“Signal transduction, as used herein, is the relaying of a signal by conversion from one physical or chemical form to another. In cell biology, this is the process by which a cell converts an extracellular signal into a response. As described above, useful antagonists are those that inhibit IL-18BP activity.

An “agonist” as defined herein, is a molecule that increases a known activity. Agonists presented herein include antibodies, fusion proteins, mimetics, peptibodies, small molecules, and other recombinant molecules. Useful agonists include those having binding affinity for one or more of the following: IL-18, IL-18R (including IL-18R α and/or IL-18R β) which can increase the immune protective functions thereof. One such category of molecules, are DR-18's, which bind to, and allow formation of the IL-18 receptor complex, but are not bound by IL-18BP, and are thus not subject to IL-18BP inhibition of IL-18 signaling.

A “peptibody” refers to molecules comprising an Fc domain and at least one peptide. Such peptibodies may be multimers or dimers or fragments thereof, and they may be derivatized. Peptibodies are described in greater detail in WO 99/25044 and WO 00/24782, which are incorporated herein by reference in their entirety. The peptide may be from the amino acid sequence of IL-18, IL-18 receptor or (IL-18R), IL-18 binding protein (IL 18BP) for inhibition or reduction of IL-18BP function or activity, and can be a substance, compound or composition designed to mimic the activity of the native molecule and augment the desired signaling pathway.

A “peptide”, as used herein refers to molecules of 1 to 40 amino acids. Alternative embodiments comprise molecules of 5 to 20 amino acids. Exemplary peptides may comprise portions of the extracellular domain of naturally occurring molecules, comprise randomized

sequences, or be rationally designed. In some embodiments, useful peptides include e.g., portions of an IL-18 sequence, or derivatives or modified versions of one or more portions of an IL-18 sequence, which inhibits the binding of IL-18 to IL-18BP to alter activity.

A "peptidomimetic" is a peptide analog that displays more favorable pharmacological properties than their prototype native peptides, such as a) metabolic stability, b) improved bioavailability, c) high receptor affinity and receptor selectivity, and d) minimal side effects. Designing peptidomimetics and methods of producing the same are known in the art (see for example, U.S. Pat. Nos. 6,407,059 and 6,420,118). Peptidomimetics may be derived from the binding site of the extra cellular domain of IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP). In alternative embodiments, a peptidomimetic comprises non peptide compounds having the same three-dimensional structure as peptides derived from IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL 18BP), and, or DR-18, or compounds in which part of a peptide from the molecules listed above is replaced by a non-peptide moiety having the same three-dimensional structure.

Embodiments of the present disclosure provide compositions and methods for inhibiting immunopathology resulting from pathogenic immune cell activity, e.g., from T cells (CD4+, CD8+, NKT, gamma-delta T), Natural Killer (NK) cells, B-cells, and Antigen presenting cells (APCs). In some embodiments, the agent can be a functional IL-18 mimetic, an agonist, and/or a decoy resistant IL-18 (DR-18).

IL-18:IL-18R biological activity, is defined herein as including, but is not limited to, binding of IL-18 to the IL-18R and activation of the IL-18R; regulation of innate and acquired immune responses; proinflammatory effects; induction of T-lymphocyte helper cell type 1 responses (Th1); enhanced cell-mediated cytotoxicity; IFN- γ induction; enhanced production of GM-CSF and IL-2; potentiation of anti-CD3 induced T-cell proliferation; increased Fas-mediated killing by natural killer cells (NK cells) and CD4+ Th1 cells; increased apoptotic death via the Fas-FasL pathway; up-regulation of FasL expression; induction of T-lymphocyte helper cell type 2 responses (Th2) in T-cells and NK cells; stimulation of basophils and mast cells to produce Th2 cytokines and histamine; induction of IgE production. Such IL-18 agonists include, but are not limited to: decoy resistant IL-18 (DR-18) antibodies directed against IL-18 that specifically bind IL-18 and partially or completely enhance binding of IL-18 to IL-18R; antibodies, fusion proteins and/or peptibodies directed against IL-18R that specifically bind IL-

18R and enhance receptor binding of IL-18 without themselves transducing a signal via IL-18R; small molecules that bind IL-18 or IL-18R that increase the interaction thereof, such as IL-18 and/or IL-18R peptidomimetics.

As used herein, when reference is made to making IL-18 agonists based on IL-18, or IL-18 receptor agonists, or IL-18 BP antagonists, it is understood that the terms IL-18 Binding Protein antagonists, and agonists of IL-18, DR-18, and IL-18 receptor also encompass fragments, variants, muteins, derivatives and fusion proteins thereof, as described in detail below. The isolation, cloning, preparation and characterization of human IL-18 receptor (referred to interchangeably as IL-18R or huIL-18R) are described in U.S. Pat. No. 6,087,116 and 6,589,764 (PCT Publication WO99/37772), which are incorporated herein by reference in their entirety.

The phrase “Antigen Presenting Cell (APC)” refers to cells that can process a protein antigen, break it into peptides, and present it in conjunction with class II MHC molecules on the cell surface where it may interact with appropriate T cell receptors. “Professional” APCs include dendritic cells, macrophages, and B cells, whereas “nonprofessional” are APCs that function in antigen presentation for only brief periods include thymic epithelial cells and vascular endothelial cells. Dendritic cells, macrophages, and B cells are the principal antigen-presenting cells for T cells, whereas follicular dendritic cells are the main antigen-presenting cells for B cells. The immune system contains three types of antigen-presenting cells, i.e., macrophages, dendritic cells, and B cells.

CD8+ regulatory T cells that are essential for maintenance of self-tolerance and prevention of autoimmune disease. In some aspects, a method for treating an autoimmune disease is provided. The method involves administering to a subject in need of such treatment an IL-18 receptor (IL-18R) agonist in an amount effective to ameliorate a symptom of the autoimmune disease.

Aspects of the disclosure describe methods of treating autoimmune diseases by isolating T cells from the subject in need of such treatment and culturing them in medium enriched with IL-18 or a mimetic or agonist thereof.

In some embodiments, the isolated cells are grown until the population of the specialized regulatory/suppressor T cells increase in number by at least 5%, 10%, at least 20%, at least 30%, at least 50%, at least 75%, at least 90%, at least 100%, or at least 200%. In some embodiments,

after culturing isolated T cells, specialized regulatory T cells may be further enriched by isolating specific populations including but not limited to CD4+CD25+ (with or without expression of CD127 or CD45RA), T regulatory cells expressing CD8+CD122+ (with or without expression of Kir or Helios) and T suppressor cells. In some embodiments, the specialized regulatory/suppressor T cells are administered to the subject by intravenous injection. The specialized regulatory/suppressor T cells are separated from the culture medium before administration to the subject. The specialized regulatory/suppressor T cells are administered to the subject in an amount effective to ameliorate a symptom of the autoimmune disease.

In some embodiments, the specialized regulatory/suppressor T cells are administered in combination with an autoimmune drug. Non-limiting examples of such drugs include methotrexate, cyclophosphamide, Imuran (azathioprine), cyclosporin, and steroid compounds such as prednisone and methylprednisolone.

SEQ ID NO: 1 (UniProt O95998)

MTMRHNWTPDLSPLWVLLLCALHVVTLVLRATPVSQTTTAATASVRSTKDPCPSQPPVF
 PAAKQCPALEVTWPEVEVPLNGTSLSCVACSRFPNFSILYWLGNFSFIEHLPGRWLWEGS
 TSRERGSTGTQLCKALVLEQLTPALHSTNFSVLDPEQVVQRHVLAQLWAGLRATL
 PPTQEALPSSHSSPQQQG

SEQ ID NO: 98 (UniProt Q14116)

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDYFGKLESKLSVIRNLNDQVLFDIQ
 GNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKE
 MNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELG
 DRSIMFTVQNE

As mentioned above, preferred antagonists comprise those that target IL-18BP. PCT Publication WO99/09063 (incorporated by reference herein) describes the IL-18BP, including useful soluble fragments thereof. One embodiment of a human IL-18BP is the “a” isoform having the polynucleotide sequence described herein as SEQ ID NO:99 and the corresponding amino acid sequence described herein as SEQ ID NO:100.

SEQ ID NO: 99

gtcgacgg-a cccccgggaa agatttaata cgactcacta tagggcgggga cagaartgat ctgtgagaga ctcatctagt
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 ggcccargt ctctgacta gtaggetca tccacagcc tctgtgtcc ctactccaa acttccagg cccttaccat
 30 gttccagaac tcccccaac ttcttgtag cagggggcac cctaaacaca caggtcccc ctgtgtacc aggggcccc
 tctccctcc tcccaacct cccctcaag atgtggaaac aaaggcaagg gcctgcagcc tgcaggcag tccactggg

agcaacaatg cctctcagct gcatggggca tgctgggagg cacaggatgg gctgcagctr cgccacgttc tctccctca
 ccctgcacag gctcagtgt acgcatggag agaatgcrag ccttagtcag gaggcaggga tctaatcera gccctgcctt
 ttcttcaga agtgcctta accaagtcac tgccctttt aagaccrctc agctttecca ctgtaacatg gactggctgc tcatccctcc
 ctgctcrga ctgagrgccc ag

5

SEQ ID NO: 100

Thr Pro Val Ser Gin Thr Thr Thr Ala Ala Thr Ala Ser Val Arg Ser Thr Lys Asp Pro Cys Pro Ser
 Gin Pro Pro Val Phe Pro Ala Ala Lys Gin Cys Pro Ala Leu Glu Val Thr

- 10 Of course, other IL-18BP isoforms that are antagonistic to IL-18 binding to IL-18R may be used.
 Such as the b, c and d isoforms. The polynucleotide and amino acid sequences for the b, c and d
 isoforms are known in the art and readily available (see for example, Kim, S.-H. et al., PNAS
 97:3 1190-1195 (2000)).

- A particularly useful form of the IL-18 binding protein, found in U.S. Patent Publication
 15 No. 2009/0297517 (incorporated herein by reference), is a fusion with an Fc domain of an
 antibody. The amino acid sequence of such a fusion protein, termed IL-18BP-Fc herein, is
 described herein as SEQ ID NO:101.

SEQ ID NO: 101

- 20 Met Arg His Asn Trp Thr Pro Asp Leu Ser Pro Leu Trp Val Leu Leu Leu Cys Ala His Val Val
 Thr Leu Leu Val Arg Ala Thr Pro Val Ser Gln Thr Thr Thr Ala Ala Thr Ala Ser Val Arg Ser Thr
 Lys Asp Pro Cys Pro Ser Gln Pro Pro Val Phe Pro Ala Ala Lys Gln Cys Pro Ala Leu Glu Val
 Thr Trp Pro Glu Val Glu Val Pro Leu Asn Gly Thr Leu Ser Leu Ser Cys Val Ala Cys Ser Arg
 Phe Pro Asn Phe Ser Ile Leu Tyr Trp Leu Gly Asn Gly Ser Phe Ile Glu His Leu Pro Gly Arg Leu
 25 Trp Glu Gly Ser Thr Ser Arg Glu Arg Gly Ser Thr Gly Thr Gln Leu Cys Lys Ala Leu Val Leu
 Glu Gln Leu Thr Pro Ala Leu His Ser Thr Asn Phe Ser Cys Val Leu Val Asp Pro Glu Gln Val
 Val Gln Arg His Val Val Leu Ala Gln Leu Trp Ala Gly Leu Arg Ala Thr Leu Pro Pro Thr Gln
 Glu Ala Leu Pro Ser Ser His Ser Ser Pro Gln Gln Gln Gly Arg Ser Cys Asp Lys Thr His Thr Cys
 Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 30 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
5 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

This protein of 422 amino acids, when expressed in a mammalian cell, is secreted from the cell.
The mature secreted form of the protein contains amino acid residues 29-422. Of these residues,
10 amino acid residues 29-192 represent the IL-18 binding protein portion of the molecule, and
amino acid residues 193-422 represent the Fc portion of the molecule. The Fc region facilitates
purification and dimerization of the fusion polypeptide. IL-18 antagonists may also comprise or
be developed from IL-18 polynucleotide and/or polypeptide sequences.

Human IL-18 has been recombinantly produced from a cloned cDNA, as described in
15 U.S. Pat. No. 5,891,663 and cloned genomic DNA, as disclosed in U.S. Pat. No. 6,060,283, each
being incorporated by reference in their entirety. The full-length cDNA sequence is described
herein as SEQ ID NO: 102 with the corresponding amino acid sequence described herein as SEQ
ID NO: 103.

20 SEQ ID NO: 102

GTAAAGTAGA AATGAATTTA TTTTCTTTG CAACTAAGT ATCTGCTTGA
GACACATCTA TCTACCATT GTCAGCTGAG GAAAAAAAAA AATGGTTCTC
ATGCTACCAA TCTGCCTTCA AAGAAATGTG GACTCAGTAG CACAGCTTTG
GAATGAAGAT GATCATAAGA GATACAAAGA AGAACCTCTA GCAAAAGATG
25 CTCTCTATG CCTTAAAAA TTCTCCAGCT CTTAGAATCT ACAAATAGA
CTTGCCTGT TTCATTGGTC CTAAGATTAG CATGAAGCCA TGGATTCTGT
TGTAGGGGGA GCGTTGCATA GGAAAAAGGG ATTGAAGCAT TAGAATTGTC
CAAAATCAGT AACACCTCCT CTCAGAAATG CTTTGGGAAG AAGCCTGGAA
GGTTCCGGGT TGGTGGTGGG GTGGGGCAGA AAATTCTGGA AGTAGAGGAG
30 ATAGGAATGG GTGGGGCAAG AAGACCACAT TCAGAGGCCA AAAGCTGAAA
GAAACCATGG CATTTATGAT GAATTCAGGG TAATTCAGAA TGGAAGTAGA

GTAGGAGTAG GAGACTGGTG AGAGGAGCTA GAGTGATAAA CAGGGTGTAG
AGCAAGACGT TCTCTCACCC CAAGATGTGA AATTTGGACT TTATCTTGGA
GATAATAGGG TTAATTAAGC ACAATATGTA TTAGCTAGGG TAAAGATTAG
TTTGTTGTAA CAAAGACATC CAAAGATACA GTAGCTGAAT AAGATAGAGA
5 ATTTTTCTCT CAAAGAAAGT CTAAGTAGGC AGCTCAGAAG TAGTATGGCT
GGAAGCAACC TGATGATATT GGGACCCCCA AC CTTCTTCA GTCTTGTACC
CATCATCCCC TAGTTGTTGA TCTCACTCAC ATAGTTGAAA ATCATCATAC
TTCCTGGGTT CATATCCCAG TTATCAAGAA AGGGTCAAGA GAAGTCAGGC
TCATTCCCTT CAAAGACTCT AATTGGAAGT TAAACACATC AATCCCCCTC
10 ATATTCCATT GACTAGAATT TAATCACATG GCCACACCAA GTGCAAGGAA
ATCTGGAAAA TATAATCTTT ATTCCAGGTA GCCATATGAC TCTTTAAAAT
TCAGAAATAA TATATTTTAA AAATATCATT CTGGCTTTGG TATAAAGAAT
TGATGGTGTG GGGTGAGGAG GCCAAAATTA AGGGTTGAGA GCCTATTATT
TTAGTTATTA CAAGAAATGA TGGTGTCATG AATTAAGGTA GACATAGGGG
15 AGTGCTGATG AGGAGCTGTG AATGGATTTT AGAAACACTT GAGAGAATCA
ATAGGACATG ATTTAGGGTT GGATTTGGAA AGGAGAAGAA AGTAGAAAAG
ATGATGCCTA CATTTTTTCAC TTAGGCAATT TGTACCATTC AGTGAAATAG
GGAACACAGG AGGAAGAGCA GGTTTTGGTG TATACAAAGA GGAGGATGGA
TGACGCATTT CGTTTTGGAT CTGAGATGTC TGTGGAACGT CCTAGTGAGG
20 ATGTCCACAA ACTCTTCTAC ATGTGGTTCT GAGTTCAGGA CACAGATTTG
GGCTGGAGAT AGAGATATTG TAGGCTTATA CATAGAAATG GCATTTGAAT
CTATAGAGAT AAAAAGACAC ATCAGAGGAA ATGTGTAAAG TGAGAGAGGA
AAAGCCAAGT ACTGTGCTGG GGGGAATACC TACATTTAAA GGATGCAGTA
GAAAGAAGCT AATAACAAC AGAGAGCAGA CTAACCAAAA GGGGAGAAGA
25 AAAACCAAGA GAATTCCACC GACTCCCAGG AGAGCATTTT AAGATTGAGG
GGATAGGTGT TGTGTTGAAT TTTGCAGCCT TGAGAATCAA GGGCCAGAAC
ACAGCTTTTA GATTAGCAA CAAGGAGTTT GGTGATCTCA GTGAAAGCAG
CTTGATGGTG AAATGGAGGC AGAGGCAGAT TGCAATGAGT GAAACAGTGA
ATGGGAAGTG AAGAAATGAT ACAGATAATT CTTGCTAAAA GCTTGGCTGT
30 TAAAAGGAGG AGAGAAACAA GACTAGCTGC AAAGTGAGAT TGGGTTGATG
GAGCAGTTTT AAATCTCAA ATAAAGAGCT TTGTGCTTTT TTGATTATGA

AAATAATGTG TTAATTGTAA CTAATTGAGG CAATGAAAAA AGATAATAAT
ATGAAAGATA AAAATATAAA AACCACCCAG AAATAATGAT AGCTACCATT
TTGATACAAT ATTTCTACAC TCCTTTCTAT GTATATATAC AGACACAGAA
ATGCTTATAT TTTTATTAAA AGGGATTGTA CTATACCTAA GCTGCTTTTT
5 CTAGTTAGTG ATATATATGG ACATCTCTCC ATGGCAACGA GTAATTGCAG
TTATATTAAG TTCATGATAT TTCACAATAA GGGCATATCT TTGCCCTTTT
TATTTAATCA ATTCTTAATT GGTGAATGTT TGTTTCCAGT TTGTTGTTGT
TATTAACAAT GTTCCCATAA GCATTCCTGT ACACCAATGT TCACACATTT
GTCTGATTTT TTCTTCAGGA TAAAACCCAG GAGGTAGAAT TGCTGGGTTG
10 ATAGAAGAGA AAGGATGATT GCCAAATTAA AGCTTCAGTA GAGGGTACAT
GCCGAGCACA AATGGGATCA GCCCTAGATA CCAGAAATGG CACTTTCTCA
TTTCCCCTTG GGACAAAAGG GAGAGAGGCA ATAACTGTGC TGCCAGAGTT
AAATTTGTAC GTGGAGTAGC AGGAAATCAT TTGCTGAAAA TGAAAACAGA
GATGATGTTG TAGAGGTCCT GAAGAGAGCA AAGAAAATTT GAAATTGCGG
15 CTATCAGCTA TGGAAGAGAG TGCTGAACTG GAAAACAAAA GAAGTATTGA
CAATTGGTAT GCTTGTAATG GCACCGATTT GAACGCTTGT GCCATTGTTC
ACCAGCAGCA CTCAGCAGCC AAGTTTGGAG TTTTGTAGCA GAAAGACAAA
TAAGTTAGGG ATTTAATATC CTGGCCAAAT GGTAGACAAA ATGAACTCTG
AGATCCAGCT GCACAGGGAA GGAAGGGAAG ACGGGAAGAG GTTAGATAGG
20 AAATACAAGA GTCAGGAGAC TGGAAGATGT TGTGATATTT AAGAACACAT
AGAGTTGGAG TAAAAGTGTA AGAAAAC TAG AAGGGTAAGA GACCGGTCAG
AAAGTAGGCT ATTTGAAGTT AACACTTCAG AGGCAGAGTA GTTCTGAATG
GTAACAAGAA ATTGAGTGTG CCTTTGAGAG TAGGTTAAAA AACAATAGGC
AACTTTATTG TAGCTACTTC TGGAACAGAA GATTGTCATT AATAGTTTTA
25 GAAAAC TAAA ATATATAGCA TACTTATTTG TCAATTAACA AAGAACTAT
GTATTTTAA ATGAGATTTA ATGTTTATTG TAG

SEQ ID NO: 103

Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala Met Lys Phe Ile Asp Asn Thr
30 Leu Tyr Phe Ile Ala Ala Glu Asp Asp Glu Glu Asn Leu Glu Ser Asp Tyr Phe Gly Lys Leu Glu
Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro

Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile
 Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser
 Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr
 Lys Ser Asp Ile Ile Phe Phe Glu Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser
 5 Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys
 Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

The amino acid sequence for human IL-18 after proteolytic processing by caspase-1 (a.k.a. IL-1
 converting enzyme, or ICE) is described herein as SEQ ID NO:104. The amino acid sequence of
 10 human IL-18 after proteolytic processing by proteinase 3 (PR3) is described herein as SEQ ID
 NO:105. Commercially available recombinant human IL-18 is available, for example, from R&D
 Systems, Minneapolis, Minn.

SEQ ID NO: 104

15 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile
 Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asp Asn Ala Pro
 Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val
 Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro
 Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
 20 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe
 Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu
 Asp

SEQ ID NO: 105

25 Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met
 Thr Asp Ser Asp Cys Arg Asp Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser
 Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn
 Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe
 Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr
 30 Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly
 Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

In certain embodiments the target of the methods and compositions described herein is human IL-18, known as Interleukin IL-18. The amino acid sequence and nucleic acid sequence for human IL-18 are publicly available, see, e.g., GENBANK Accession No. NP_001230140.1

5 (SEQ ID NO: 107) and NM_001243211.2 (SEQ ID NO: 106) (both of which are incorporated by reference herein).

SEQ ID NO: 106

cctttgctcc cctggcgact gcctggacag tcagcaagga attgtctccc agtgcatttt gccctcctgg ctgccaactc
 10 tggtgctctaa agcggctgcc acctgtgca gtctacacag ctfcgggaag aggaaaggaa cctcagacct tccagatcgc
 ttctctcgc aacaaactat ttgtcgcagg aataaagatg gctgctgaac cagtagaaga caattgcac aactttgtgg caatgaaatt
 tattgacaat acgctttact ttatagaaaa cctggaatca gattactttg gcaagcttga atctaaatta tcagtcataa gaaatttgaa
 tgaccaagtt ctcttcattg accaaggaaa tcggcctcta ttgaagata tgactgattc tgactgtaga gataatgcac cccggaccat
 atttattata agtatgtata aagatagcca gcctagaggt atggctgtaa ctatctctgt gaagtgtgag aaaatttcaa ctctctcctg
 15 tgagaacaaa attatttct ttaaggaaat gaatcctcct gataacatca aggatacaaa aagtgcacac atattcttcc agagaagtgt
 cccaggacat gataataaga tgcaatttga atcttcacac tacgaaggat actttctagc ttgtgaaaaa gagagagacc ttttaaaact
 cattttgaaa aaagaggatg aattggggga tagatctata atgttactg tcaaaaacga agactagcta ttaaaatttc atgccggggcg
 cagtggctca cgctgtaat cccagccctt tgggaggctg aggcggggcag atcaccagag gtcagggtgt caagaccagc
 ctgaccaaca tgggtgaaacc tcactctac taaaaataca aaaaattagc tgagtgtagt gacgcattgcc ctcaatccca
 20 gctactcaag aggtgagggc aggagaatca ctgcactcc ggaggtagag gttgtggtga gccgagattg caccattgcg
 ctctagcctg ggcaacaaca gcaaaactcc atctcaaaaa ataaaataaa taaataaaca aataaaaaat tca

SEQ ID NO: 107

MAAEPVEDNC INFVAMKFID NTLFYFIENLE SDYFGKLESK LSVIRNLNDQ
 25 VLFDIQGNRP LFEDMTSDSC RDNAPRTIFI ISMYKDSQPR GMAVTISVKC
 EKISTLSCEN KIISFKEMNP PDNIKDTKSD IIFQRSVPG HDNKMQFESS SYEGYFLACE
 KERDLFKLIL KKEDELGDRS IMFTVQNED

In certain embodiments the target of the methods and compositions described herein is human
 30 DR-18, also known as decoy resistant IL-18. The amino acid sequence and nucleic acid sequence
 for active (i.e., proteolytically processed) human DR-18 are publicly available, see, e.g. U.S.

Patent Publication No. 2019/0070262 A1. This publication contains the below sequence for wild type hIL-18 (SEQ ID NO: 108)

SEQ ID NO: 108

5 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile
Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg
Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys
Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp
Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met
10 Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys
Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

DR-18 polypeptides bind to, and signal through formation of, the IL-18R α (IL-18
receptor α) and IL-18R β (IL-18 receptor β) receptor complex. DR-18 polypeptides display
substantially reduced binding to IL-18BP, such as substantially reduced binding to IL-18BP
15 relative to wild-type (WT) IL-18 (SEQ ID NO: 108), or do not bind to IL-18BP (i.e., as
compared to the binding of IL-18BP to WT IL-18 (SEQ ID NO: 108)). In some embodiments,
the DR-18 polypeptide employed binds to IL-18R α and does not bind to IL-18BP. In some
embodiments, the DR-18 polypeptide employed binds to IL-18R α and has reduced binding to IL-
18BP relative to WT IL-18.

20 Useful DR-18 polypeptides include, but are not limited to, e.g., those described in PCT
Pub. No. WO2019/051015A1 and US Pat. Pub. No. US20210015891A1, the disclosures of
which are incorporated herein by reference in their entirety.

In some embodiments, the DR-18 polypeptide comprises a modified IL-18 polypeptide
comprising: (i) an amino acid sequence having 85% or more (e.g., at least 90%, 91%, 92%, 93%,
25 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity with the IL-18 variant amino
sequence set forth in any one of SEQ ID NO: 2-63; with or without (ii) mutations at amino acid
positions Cysteine-38 and Cysteine-68 relative to WT IL-18 as set forth in SEQ ID NO: 108.
DR-18 polypeptides, and compositions comprising DR-18 polypeptides, useful in the herein
described methods are described in more detail below. Any of the herein described DR-18
30 polypeptides may find use in the methods of the present disclosure. In some instances, the
methods will employ a DR-18 polypeptide (or a DR-18 composition comprising such a

polypeptide) comprising an amino acid sequence having 85% or more (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity with the IL-18 variant amino sequence set forth in any one of SEQ ID NO: 2-63. In some instances, the methods will employ a DR-18 polypeptide (or a DR-18 composition comprising such a polypeptide)

5 comprising an amino acid sequence having 85% or more (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity with the IL-18 variant amino sequence set forth in SEQ ID NO: 20, 63, 7, 17-19, 21, 22, 55-57, and 62.

In some instances, a useful DR-18 polypeptide comprises an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%
10 sequence identity with SEQ ID NO:108 and/or at least one of SEQ ID NOs: 7, 17-22, 55-57, 62 and 63. In some instances, a useful DR-18 polypeptide comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, or 15 mutated residues relative to SEQ ID NO:108. In some instances, a useful DR-18 polypeptide comprises up to 12, 11, or 10 mutated residues relative to SEQ ID NO:108.

In some instances, a useful DR-18 polypeptide comprises from 1 to 12, from 2 to 12,
15 from 3 to 12, from 4 to 12, from 5 to 12, from 6 to 12, from 7 to 12, from 8 to 12, from 9 to 12, from 10 to 12, from 1 to 10, from 2 to 10, from 3 to 10, from 4 to 10, from 5 to 10, from 6 to 10, from 7 to 10, or from 8 to 10 mutated residues relative to SEQ ID NO:108.

In some instances, a useful DR-18 polypeptide comprises an amino acid sequence of any one of SEQ ID NOs: 7, 17-22, 55-57, 62 and 63 with 0, 1, 2, 3, 4, 5, or 6 additional mutated
20 residues relative to SEQ ID NO:108. DR-18 polypeptides may or may not include (or be modified to include or exclude) one or more cysteine mutations relative to WT IL-18. For example, a stabilized IL-18 polypeptide can include mutations of two cysteine residues (e.g., C38 and C68) relative to human WT IL-18 (SEQ ID NO:108). In some cases, the mutation is a C to S substitution and as such the stabilized IL-18 polypeptide can in some cases comprise the
25 mutations C38S and C68S. In some cases a stabilized IL-18 polypeptide comprises the mutation pair C38S/C68S, C38S/C68G, C38S/C68A, C38S/C68V, C38S/C68D, C38S/C68E, or C38S/C68N (e.g., in some cases C38S/C68G, C38S/C68A, C38S/C68V, C38S/C68D, C38S/C68E, or C38S/C68N; in some cases C38S/C68S, C38S/C68G, C38S/C68A, C38S/C68D, or C38S/C68N; and in some cases C38S/C68G, C38S/C68A, C38S/C68D, or C38S/C68N).

30 In some instances, a useful DR-18 polypeptide comprises one or more mutations at positions Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110,

N111, M113, V153, and N155 relative to the WT IL-18 amino acid sequence set forth in SEQ ID NO:108, including e.g., at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, or at least 12 of the previously listed mutated positions. Accordingly, useful DR-18 polypeptides can have one or any combination of mutations at the listed positions, with or without additional mutated residues at other positions. For example, useful polypeptides can have a mutation at position Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110, N111, M113, V153, or N155 only; mutations at all positions Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110, N111, M113, V153, and N155; or any combinations having more than one but less than all (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19) mutations at these positions.

A DR-18 polypeptide may comprise at least 12 mutations, at least 11 mutations, at least 10 mutations, at least 9 mutations, at least 8 mutations, at least 7 mutations, at least 6 mutations, at least 5 mutations, at least 4 mutations, at least 3 mutations, at least 2 mutations, or at least 1 mutation relative to the wild-type IL-18 amino acid sequence set forth in SEQ ID NO:108, optionally wherein the at least 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mutations are at positions selected from Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110, N111, M113, V153, and N155. In some instances, the residue at position (pos.) 1 is Y, R or H; the residue at pos. 5 is L, H, I or Y; the residue at pos. 8 is K, Q or R; the residue at pos. 38 is C or S; the residue at pos. 51 is M, T, K, D, N, E or R; the residue at pos. 53 is K, R, G, S or T; the residue at pos. 55 is S, K or R; the residue at pos. 56 is Q, E, A, R, V, G, K, L or R; the residue at pos. 57 is P, L, G, A or K; the residue at pos. 59 is G, A or T; the residue at pos. 60 is M, K, Q, R or L; the residue at pos. 68 is C, S, G, A, V, D, E or N; the residue at pos. 76 is C or S; the residue at pos. 77 is E or D; the residue at pos. 103 is Q, E, K, P, A or R; the residue at pos. 105 is S, D, N, R, K or A; the residue at pos. 110 is D, K, H, N, Q, E, S or G; the residue at pos. 111 is N, H, Y, D, R, S or G; the residue at pos. 113 is M, V, R, T or K; the residue at pos. 127 is C or S; the residue at pos. 153 is V, I, T or A; and the residue at pos. 155 is N, K or H.

In some instances, the DR-18 polypeptide comprises one or more substitutions relative to WT human IL-18 (SEQ ID NO: 108), including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more substitutions selected from: Y1H, Y1R, L5H, L5I, L5Y, K8Q, K8R, M51T, M51K, M51D, M51N, M51E, M51R, K53R, K53G, K53S, K53T, S55K, S55R, Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, Q56L, P57L, P57G, P57A, P57K, G59T, G59A, M60K, M60Q, M60R, M60L, E77D, Q103E,

Q103K, Q103P, Q103A, Q103R, S105R, S105D, S105K, S105N, S105A, D110H, D110K, D110N, D110Q, D110E, D110S, D110G, N111H, N111Y, N111D, N111R, N111S, N111G, M113V, M113R, M113T, M113K, V153I, V153T, V153A, N155K, and N155H.

In some instances, the DR-18 polypeptide comprises one or more substitutions relative to WT human IL-18 (SEQ ID NO: 108) such that, e.g., the amino acid at pos. 1 is not Y and, e.g., may be R or H; the amino acid at pos. 5 is not L and, e.g., may be H, I or Y; the amino acid at pos. 8 is not K and, e.g., may be Q or R; the amino acid at pos. 38 is not C and, e.g., may be S; the amino acid at pos. 51 is not M and, e.g., may be T, K, D, N, E or R; the amino acid at pos. 53 is not K and, e.g., may be R, G, S or T; the amino acid at pos. 55 is not S and, e.g., may be K or R; the amino acid at pos. 56 is not Q and, e.g., may be E, A, R, V, G, K, L or R; the amino acid at pos. 57 is not P and, e.g., may be L, G, A or K; the amino acid at pos. 59 is not G and, e.g., may be A or T; the amino acid at pos. 60 is not M and, e.g., may be K, Q, R or L; the amino acid at pos. 68 is not C and, e.g., may be S, G, A, V, D, E or N; the amino acid at pos. 76 is not C and, e.g., may be S; the amino acid at pos. 77 is not E and, e.g., may be D; the amino acid at pos. 103 is not Q and, e.g., may be E, K, P, A or R; the amino acid at pos. 105 is not S and, e.g., may be D, N, R, K or A; the amino acid at pos. 110 is not D and, e.g., may be K, H, N, Q, E, S or G; the amino acid at pos. 111 is not N and, e.g., may be H, Y, D, R, S or G; the amino acid at pos. 113 is not M and, e.g., may be V, R, T or K; the amino acid at pos. 127 is not C and, e.g., may be S; the amino acid at pos. 153 is not V and, e.g., may be an I, T or A; and/or the amino acid at pos. 155 is not N and, e.g., may be K or H.

In some instances, the DR-18 polypeptide comprises substitution mutations, relative to WT human IL-18 as set forth in SEQ ID NO:108, at positions: (i) M51, M60, S105, D110, and N111; (ii) M51, S55, G59, M60, S105, D110, N111, and V153; (iii) Y1, M51, M60, S105, D110, and N111; (iv) Y1, M51, K53, M60, S105, D110, and N111; (v) K8, M51, S55, G59, M60, S105, D110, and N155; (vi) K8, M51, S55, G59, M60, S105, D110, N111, and V153; (vii) L5, M51, K53, M60, S105, D110, and V153; (viii) L5, M51, S55, G59, M60, S105, D110, N111, and N155; (ix) L5, M51, S55, M60, Q103, S105, D110, N111, and V153; (x) L5, M51, S55, M60, S105, D110, N111, V153, and N155; (xi) L5, M51, S55, G59, M60, S105, D110, N111, V153, and N155; (xii) L5, K8, M51, S55, M60, S105, N111, V153, and N155; (xiii) L5, K8, M51, K53, M60, S105, D110, N111, and N155; (xiv) Y1, L5, M51, K53, M60, S105, D110, and N155; (xv) Y1, M51, K53, G59, M60, S105, D110, N111, V153, and N155; (xvi) Y1, K8,

M51, K53, M60, Q103, S105, D110, N111, and N155; (xvii) Y1, K8, M51, M60, S105, D110, and N111; (xviii) Y1, L5, M51, K53, M60, Q103, S105, D110, and N111; (xix) Y1, K8, M51, K53, G59, M60, Q103, S105, D110, N111, V153, and N155; (xx) Y1, K8, M51, K53, G59, M60, S105, D110, N111, and N155; (xxi) Y1, K8, M51, G59, M60, Q103, S105, D110, N111, V153, and N155; (xxii) Y1, L5, M51, G59, M60, E77, S105, D110, and N111; (xxiii) M51, Q56, P57, M60, Q103, S105, D110, N111, and M113; (xxiv) M51, Q56, P57, M60, Q103, S105, D110, and M113; (xxv) M51, K53, Q56, P57, M60, D110, and N111; (xxvi) M51, K53, Q56, P57, M60, Q103, S105, D110, N111, and M113; (xxvii) M51, K53, Q56, M60, Q103, S105, D110, N111, and M113; (xxviii) M51, K53, Q56, P57, Q103, S105, D110, N111, and M113; (xxix) M51, K53, Q56, P57, M60, S105, D110, and N111; (xxx) M51, K53, Q56, P57, M60, Q103, D110, N111, and M113; (xxxi) M51, Q56, P57, M60, Q103, D110, N111, and M113; or (xxxii) M51, K53, Q56, S105, D110, and N111.

In some instances, the DR-18 polypeptide comprises substitution, relative to WT human IL-18 as set forth in SEQ ID NO:108, of: (i) M51T, M60K, S105D, D110K, and N111H; (ii) M51T, S55K, G59A, M60K, S105D, D110K, N111H, and V153I; (iii) Y1R, M51T, M60K, S105D, D110K, and N111H; (iv) Y1R, M51T, K53R, M60K, S105N, D110K, and N111Y; (v) K8Q, M51T, S55K, G59T, M60K, S105R, D110H, and N155K; (vi) K8R, M51K, S55K, G59A, M60Q, S105D, D110K, N111H, and V153I; (vii) K8R, M51D, S55K, G59A, M60X, S105D, D110K, N111H, and V153I; (viii) L5H, M51T, K53R, M60K, S105D, D110N, and V153T; (ix) L5I, M51K, S55K, G59A, M60Q, S105K, D110Q, N111H, and N155K; (x) L5I, M51T, S55R, M60K, Q103E, S105D, D110H, N111H, and V153I; (xi) L5I, M51T, S55K, M60K, S105D, D110K, N111H, V153T, and N155H; (xii) L5I, M51T, S55K, G59A, M60K, S105R, D110H, N111H, V153I, and N155K; (xiii) L5I, K8R, M51T, S55K, M60K, S105D, N111Y, V153I, and N155K; (xiv) L5Y, K8R, M51T, K53R, M60K, S105D, D110E, N111H, and N155K; (xv) Y1H, L5Y, M51T, K53R, M60K, S105D, D110H, and N155K; (xvi) Y1R, M51T, K53R, G59A, M60K, S105D, D110Q, N111H, V153A, and N155K; (xvii) Y1R, K8R, M51D, K53R, M60R, Q103K, S105N, D110K, N111Y, and N155H; (xviii) Y1R, K8R, M51N, K53R, M60Q, Q103K, S105R, D110N, N111H, and N155K; (xix) Y1R, K8R, M51T, M60K, S105D, D110K, and N111H; (xx) Y1R, L5H, M51T, K53R, M60K, Q103E, S105N, D110K, and N111Y; (xxi) Y1R, K8R, M51T, K53R, G59A, M60K, Q103E, S105D, D110Q, N111H, V153I, and N155X; (xxii) Y1R, K8R, M51T, K53R, G59T, M60K, S105N, D110H, N111D, and N155H; (xxiii) Y1R,

K8R, M51T, G59A, M60K, Q103E, S105D, D110Q, N111H, V153I, and N155K; (xxiv) Y1R, L5Y, M51T, G59T, M60K, E77D, S105D, D110K, and N111H; (xxv) Y1R, K8R, M51T, K53R, G59T, M60K, S105K, D110N, N111H, and N155K; (xxvi) M51E, Q56E, P57L, M60R, Q103P, S105A, D110N, N111R, and M113V; (xxvii) M51K, Q56A, P57G, M60L, Q103E, S105D, D110S, and M113V; (xxviii) M51K, K53G, Q56A, P57A, M60L, D110K, and N111R; (xxix) M51K, K53G, Q56R, P57G, M60L, Q103E, S105D, D110N, N111S, and M113R; (xxx) M51K, K53G, Q56V, M60L, Q103A, S105A, D110S, N111R, and M113T; (xxxi) M51K, K53S, Q56G, P57A, M60L, Q103A, S105A, D110G, N111R, and M113T; (xxxii) M51K, K53S, Q56K, P57A, Q103A, S105D, D110S, N111S, and M113R; (xxxiii) M51K, K53S, Q56L, P57A, M60L, S105D, D110S, and N111R; (xxxiv) M51K, K53S, Q56R, P57A, M60L, S105N, D110G, and N111R; (xxxv) M51K, K53S, Q56R, P57A, M60L, Q103A, D110G, N111R, and M113T; (xxxvi) M51K, K53S, Q56R, P57A, M60L, Q103A, S105D, D110S, N111G, and M113R; (xxxvii) M51K, K53T, Q56R, M60L, Q103E, S105D, D110S, N111S, and M113K; (xxxviii) M51K, K53T, Q56R, P57A, Q103E, S105D, D110N, N111D, and M113R; (xxxix) M51R, Q56G, P57K, M60L, Q103R, D110S, N111R, and M113V; (xl) M51K, K53G, Q56G, P57A, M60L, Q103E, S105D, D110S, N111G, and M113V; or (xli) M51K, K53G, Q56R, S105A, D110N, and N111R.

In some instances, the engineered IL-18 polypeptide is a DR IL-18 polypeptide that comprises 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 7, 17-22, 55-57, 62 and 63. In some instances, the engineered IL-18 polypeptide is a DR IL-18 polypeptide that comprises 100% sequence identity to SEQ ID NO: 20.

Useful DR-18 polypeptides include those listed by sequence in the following table:

DR-18 Amino Acid Sequence	SEQ ID NO.:
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSSSENKISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNED	2
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSSSENKISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNED	3

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	4
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	5
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	6
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	7
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	8
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYE GYFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	9
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	10
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	11
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	12
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	13
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKSEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	14
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSSSENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	15

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLASEKERDLFKLILKKEDELGDRSIMFTVQNE	16
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKGEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	17
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKA EKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	18
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKVEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	19
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKDEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	20
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKEEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	21
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYSDSLARGL AVTISVKNEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	22
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	23
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDKQPRAK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQNE	24
RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	25
RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRNVPGHKYKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	26
YFGKLESQLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDKQPRTK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRRVPGHHNKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	27

YFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYKDKQPRAQ AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQNED	28
YFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISDYKDKQPRAX AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQNED	29
YFGKHESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHNNKMQLFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTTQNE	30
YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYKDKQPRAQ AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRKVPGHQHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	31
YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDRQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHKKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQNED	32
YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDKQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTTQHED	33
YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDKQPRAK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRRVPGHHKKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQKED	34
YFGKIESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDKQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHDYKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQKED	35
YFGKYESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHEHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	36
HFGKYESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHHNNKMQLFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	37
RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRAK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHQHKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTAQKED	38
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISDYRDSQPRGR AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFKRNVPGHKYKMQFESSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQHED	39

RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISNYRDSQPRGQ AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFKRRVPGHNHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	40
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	41
RFGKHESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRGK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFERNVPGHKYKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	42
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRAK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHQHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQXED	43
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRTK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRNVPGHHDKMQLFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQHED	44
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDSQPRAK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHQHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTIQKED	45
RFGKYESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYKDSQPRTK AVTISVKCEKISTLSCDNKIISFKEMNPPDNIKDTKSDIIFQRDVPGHKHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	46
RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISTYRDSQPRTK AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRKVPGHNHKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQKED	47
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISEYKDSELRGR AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFPRAVPGHNRKVQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	48
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYKDSAGRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHSNKVQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	49
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYGDSAARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHKRKMQLFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	50
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYGDSRGRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHNSKRQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	51

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYGDSVPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFARAVPGHSRKTQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	52
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSGARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFARAVPGHGRKTQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	53
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSKARGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFARDVPGHSSKRQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	54
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSLARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRDVPGHSRKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	55
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSRARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRNVPGHGRKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	56
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSRARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFARSVPGHGRKTQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	57
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDSRARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFARDVPGHSGKRQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	58
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYTDSRPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFERDVPGHSSKKQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	59
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYTDSRARGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFERDVPGHNDKRQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	60
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISRYKDSGKRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFRRSVPGHSRKVQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	61
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYGD SGARGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFERDVPGHSGKVQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	62
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYGDSRPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRAVPGHNRKMQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	63

Also useful, in some embodiments, are nucleic acids that encode a DR-18 polypeptide, including e.g., one of the DR-18 polypeptides included above. For example, in some instances, the methods of the present disclosure may include administering to a subject in need thereof, a nucleic acid (e.g., a mRNA or a DNA) that encodes the DR-18 polypeptide. Such nucleic acids may be administered “naked” (e.g., in the case of naked linear or circular DNA or RNA) or encased or encapsulated, e.g., in a viral particle or non-viral vector. Useful viral and non-viral vectors, and useful components thereof, include e.g., adenoviral vectors, AAV vectors, lentiviral vectors, lipid nanoparticle vectors, and the like. In some instances, the methods of the present disclosure may include administering to a subject in need thereof, a cell (such as e.g., a CD8+ T cell) that comprises an introduced nucleic acid (e.g., a mRNA or a DNA) that encodes the DR-18 polypeptide. Useful cells may be autologous or allogeneic with respect to the subject being treated. In such cells, such introduced nucleic acids may be integrated into the genome of the cell (e.g., through the use of an integrating vector, site directed nucleases, homologous recombination, or any combination thereof or the like) or may be extrachromosomal. Cells engineered to contain a sequence encoding a DR-18 polypeptide and express the DR-18, (constitutively, conditionally or inducibly) may be referred to as “armored with DR-18” or a “DR-18 armored cell”.

In one embodiment, IL-18 agonists are polypeptide mimetics, such as, but not limited to peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of IL-18 (listed above). Polypeptide mimetics may be developed through techniques known in the art, such as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. In one aspect, an IL-18 polypeptide mimetic based on the amino acid sequence of IL-18 will bind to IL-18 receptor without activating the pro-inflammatory signaling pattern of the IL-18 receptor and promote its immune protect function. As such, IL-18 polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with inhibiting disease associated immunopathology resulting from pathogenic, autoreactive CD4+ T cell signaling.

Biological activity, in this instance, is the capacity to bind its cognate partner. The present disclosure additionally provides for the use of IL-18 agonists in the manufacture of a medicament for the treatment of immunopathology resulting from CD4+ T cell disease symptoms resulting from aberrant immune cell signaling. Further provided is the use of

polynucleotides encoding IL-18 or variants thereof for increasing unopposed IL-18, in the manufacture of a medicament for the treatment of inhibiting immunopathology resulting from pathogenic or autoreactive CD4+ T cell activity.

Various means for attaching chemical moieties useful for increasing bio-availability and/or pharmacokinetic half-life are currently available, see, e.g., Patent Cooperation Treaty (“PCT”) International Publication No. WO 96/11953, entitled “N-Terminally Chemically Modified Protein Compositions and Methods,” herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water soluble polymers to the N-terminus of proteins.

A preferred polymer vehicle is polyethylene glycol (PEG). The PEG group may be of any convenient molecular weight and may be linear or branched. The average molecular weight of the PEG will preferably range from about 2 kiloDalton (“kD”) to about 100 kD, more preferably from about 5 kD to about 50 kD, most preferably from about 5 kD to about 10 kD. The PEG groups will generally be attached to the compounds of the invention via acylation or reductive alkylation through a reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are “preactivated” with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

Polysaccharide polymers are another type of water-soluble polymer which may be used for protein modification. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by α -1,6 linkages. The dextran itself is available in many molecular weight ranges and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water-soluble polymer for use in the present disclosure as a vehicle by itself or in combination with another vehicle (e.g., Fc). See, for example, WO 96/11953 and

WO 96/05309. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported; see, for example, European Patent Publication No. 0 315 456, which is hereby incorporated by reference in its entirety. Dextran of about 1 kD to about 20 kD is preferred when dextran is used as a vehicle in accordance with the present disclosure.

5

“Decoy-to-the-decoy” (D2D) engineered IL-18 polypeptides as IL-18BP Antagonists

As described above, wild type IL-18 binds to both IL-18R (to signal through the receptor) and IL-18BP (which inhibits IL-18 by preventing it from binding to IL-18R). DR-18 variants bind to IL-18R but have reduced binding to (and in some cases do not bind to) IL-18BP. Also
10 useful are so called "decoy-to-the-decoy IL-18" ("D2D IL- 18") variants. Such D2D variants bind to IL-18BP but have reduced binding to (and in some cases do not bind to) IL-18R. Binding affinity may be comparable binding affinity, such as comparable binding affinity to IL-18R as wild type IL-18, or may be compared to wild type IL-18, such as exhibiting decreased binding affinity as compared to wild type IL-18.

15 Useful D2D polypeptides include, but are not limited to, e.g., those described in Clark et al.²⁹, PCT Pub. No. WO2019/051015A1 and US Pat. Pub. No. US20210015891A1, the disclosures of which are incorporated herein by reference in their entirety. D2D polypeptides may or may not include (or be modified to include or exclude) one or more cysteine mutations relative to WT IL-18. For example, a stabilized IL-18 polypeptide can include mutations of two
20 cysteine residues (e.g., C38 and C68) relative to human WT IL-18 (SEQ ID NO:108). In some cases, the mutation is a C to S substitution and as such the stabilized IL-18 polypeptide can in some cases comprise the mutations C38S and C68S. In some cases a stabilized IL-18 polypeptide comprises the mutation pair C38S/C68S, C38S/C68G, C38S/C68A, C38S/C68V, C38S/C68D, C38S/C68E, or C38S/C68N (e.g., in some cases C38S/C68G, C38S/C68A,
25 C38S/C68V, C38S/C68D, C38S/C68E, or C38S/C68N; in some cases C38S/C68S, C38S/C68G, C38S/C68A, C38S/C68D, or C38S/C68N; and in some cases C38S/C68G, C38S/C68A, C38S/C68D, or C38S/C68N).

In some instances, useful D2D's include a D2D IL-18 polypeptide that comprises 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 64-97. In some
30 instances, useful D2D's include a D2D IL-18 polypeptide that comprises at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from SEQ ID NOs: 64-97.

Useful D2D polypeptides include those listed by sequence in the following table:

D2D Amino Acid Sequence	SEQ ID NO.:
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFKDMTASDCRANAPRTIFIISFYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGADNKFQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	64
DFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMTDNPCRSNAPRTIFIISFYKDSQPRGIA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGPDNKMQFESSSYEGY FLACEKERDLFKLILKKEDELGDRSIMFTVQNED	65
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMEASPCRDNAPRTIFIISFYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	66
LFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSSPCRSRAPRTIFIISFYKDSQPRGFA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKFQFESSSYEGYF LACEKERDLFKLILKKEDELGDRSIMFTVQNED	67
HFGKLESKLSVIRNLNDQVLFIDQGNRPLFTDMESKPCRDSAPRTIFIISMYKDSQPRGIA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKFQFESSSYEGYF LACEKERDLFKLILKKEDELGDRSIMFTVQNED	68
YFGKLESKLSVIRNLNRQVLFIDQGNRPLFTDMTYKDCRDNAPRTIFIISFYKDSQPRGF AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGADNKFQFESSSYEGY FLACEKERDLFKLILKKEDELGDRSIMFTVQNED	69
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMEASPCRDNAPRTIFIISFYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGADNKLQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	70
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTSSDCRDKAPRTIFIISFYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGPDNKFQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	71
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMESNRCDNAPRTIFIISMYKDSQPRGF AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKFQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	72
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTASPCRDNAPRTIFIISFYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKFQFESSSYEG YFLACEKERSLFLKILKKEDELGDRSIMFTVQNED	73

DFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKS NVCRANAPRTIFIISMYKDSQPRG MAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGPDNKLQFESSSYE GYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	74
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMEASPCRAKAPRTIFIISIYKDSQPRGFA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKFQFESSSYEGY FLACEKERSLFLKLILKKEDELGDRSIMFTVQNE	75
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMASNRCRANAPRTIFIISMYKDSQPRGF AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGPDNKFQFESSSYEGY FLACEKERDLFKLILKKEDELGDRSIMFTVQNE	76
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKAKACRSNAPRTIFIISFYKDSQPRGF AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGADNKIQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	77
HFGKLESKLSVIRNLNHQVLFIDQGNRPLFTDMADNACRDNAPRTIFIISFYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGDDNKMQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	78
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMKSNLCRSNAPRTIFIISFYKDSQPRGIA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGDDNKKIQFESSSYEGYF LACEKERDLFKLILKKEDELGDRSIMFTVQNE	79
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFRDMAASHCRDSAPRTIFIISIYKDSQPRGFA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKKIQFESSSYEGYF LACEKERDLFKLILKKEDELGDRSIMFTVQNE	80
YFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMASNPCRYKAPRTIFIISMYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGADNKLQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	81
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMASNHCRYNAPRTIFIISMYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGADNKKIQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	82
HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTDNPCRSRAPRTIFIISFYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKFQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	83
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTASHCRSSAPRTIFIISLYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKFQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	84
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMEYRLCRANAPRTIFIISFYKDSHPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGDDNKLQFESSSYEG YFLACEKERDLFKLILKKEDELGDRSIMFTVQNE	85

YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMESSLCRDNAPRTIFIISLYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGADNKFQFESSSYEG YFLACEKERSLFLKILKKEDELGDRSIMFTVQNE	86
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFKDMEANDCRSSAPRTIFIISIYKDSQPRGLA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGADNKMVFESSSYEGY FLACEKERDLFLKILKKEDELGDRSIMFTVQNE	87
DFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKASACRANAPRTIFIISMYKDSQPRG LAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKFQFESSSYE GYFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	88
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMTAKHCRARAPRTIFIISFYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGADNKFQFESSSYEG YFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	89
FFGKFESKLSVIRNLNGQVLFIDQGNRPLFTDMESKDCRDRAPRTIFIISFYKDSQPRGLA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKLQFESSSYEGY FLACEKERDLFLKILKKEDELGDRSIMFTVQNE	90
FFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMASNHCRANAPRTIFIISLYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKMVFESSSYEG YFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	91
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSKRCRDNAPRTIFIISLYKDSQPRGF AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKIQFESSSYEGY FLACEKERDLFLKILKKEDELGDRSIMFTVQNE	92
LFGKHESKLSVIRNLNGQVLFIDQGNRPLFGDMESSPCRYNAPRTIFIISFYKDSQPRGLA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFIRSVPGHDNKMVFESSSYEGY FLACEKERDLFLKILKKEDELGDRSIMFTVQNE	93
YFGKLESKLSVIRNLNAQVLFIDQGNRPLFTDMTASPCRSSAPRTIFIISLYKDSQPRGLA VTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGPDNKIQFESSSYEGYF LACEKERDLFLKILKKEDELGDRSIMFTVQNE	94
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSDSCRDNAPRTIFIISMYKDSQPRG MAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKMVFESSSY EGYFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	95
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSSDCRDNAPRTIFIISFYKDSQPRGM AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKMVFESSSYEG YFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	96
YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMESSDCRDNAPRTIFIISFYKDSQPRGL AVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFLRSVPGHDNKMVFESSSYEG YFLACEKERDLFLKILKKEDELGDRSIMFTVQNE	97

Also useful, in some embodiments, are nucleic acids that encode a D2D polypeptide, including e.g., one of the D2D polypeptides included above. For example, in some instances, the methods of the present disclosure may include administering to a subject in need thereof, a nucleic acid (e.g., a mRNA or a DNA) that encodes the D2D polypeptide. Such nucleic acids may be administered “naked” (e.g., in the case of naked linear or circular DNA or RNA) or encased or encapsulated, e.g., in a viral particle or non-viral vector. Useful viral and non-viral vectors, and useful components thereof, include e.g., adenoviral vectors, AAV vectors, lentiviral vectors, lipid nanoparticle vectors, and the like. In some instances, the methods of the present disclosure may include administering to a subject in need thereof, a cell (such as e.g., a CD8+ T cell) that comprises an introduced nucleic acid (e.g., a mRNA or a DNA) that encodes the D2D polypeptide. Useful cells may be autologous or allogeneic with respect to the subject being treated. In such cells, such introduced nucleic acids may be integrated into the genome of the cell (e.g., through the use of an integrating vector, site directed nucleases, homologous recombination, or any combination thereof or the like) or may be extrachromosomal. Cells engineered to contain a sequence encoding a D2D polypeptide and express the D2D, (constitutively, conditionally or inducibly) may be referred to as “armored with D2D” or a “D2D armored cell”.

Antibodies as IL-18, DR-18 and IL-18R Agonists and IL-18BP Antagonists.

Antibodies can be designed to act as IL-18, IL-18R agonists and IL-18BP antagonists include antibodies that specifically bind these molecules and modulate their activity in a therapeutically beneficial way. More specifically, IL-18 agonists include antibodies directed against IL-18 that specifically bind IL-18 and enhance binding of IL-18 to IL-18R; antibodies directed against IL-18R that specifically bind IL-18R and stimulate receptor binding of IL-18 without themselves transducing a signal via IL-18R; antibodies directed against IL-18R that specifically bind IL-18R and mimic the function of IL-18 (30), and the like.

IL-18, IL-18R, and IL-18BP, as well as fragments, variants, muteins, derivatives and fusion proteins thereof, as set forth above, can be employed as “immunogens” in producing antibodies that may be used in the diagnosis and inhibition of immunopathology resulting from pathogenic or autoreactive CD4+ T cell activity. In making IL-18 or IL-18R agonists or IL-18BP

antagonists in the form of antibodies, when reference is made to such molecules, it is understood to also encompass fragments, variants, muteins, derivatives and fusion proteins thereof.

Each of IL-18, IL-18R, DR-18, and IL-18BP contain antigenic determinants or epitopes that elicit the formation of antibodies. These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon polypeptide folding. Epitopes can be identified by any of the methods known in the art. Additionally, epitopes from the polypeptides of the disclosure can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

Antibodies to IL-18, IL-18R, DR-18, IL-18BP, can conveniently be generated against a recombinantly produced form of the proteins described above and provided in the respective sequence identifier numbers. IL-18 agonists can be antibodies that include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab)₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, single domain antibodies (sdAbs) and epitope-binding fragments of any of the above. Such antibodies can be utilized in methods of inhibiting immunopathology resulting from pathogenic or autoreactive CD4⁺ T cell signaling.

Both polyclonal and monoclonal antibodies to the target molecules can be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); Kohler and Milstein, (U.S. Pat. No. 4,376,110); the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983); and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96).

Methods of making humanized monoclonal antibodies are well known, and include for example those described in U.S. Pat. No. 5,585,089 (Protein Design: C L Queen et al.;

“Humanized Immunoglobulins”), U.S. Pat. No. 5,565,332 (“Production of Chimeric Antibodies-A Combinatorial Approach”), U.S. Pat. No. 5,225,539 (Med Res Council: G P Winter; “Recombinant Altered Antibodies And Methods Of Making Altered Antibodies”), U.S. Pat. No. 5,693,761-762 (Protein Design: C L Queen et al.; “Polynucleotides Encoding Improved Humanized Immunoglobulins”, and “Humanized Immunoglobulins”), and U.S. Pat. No. 5,530,101 (Protein Design: C L Queen et al.; “Humanized Immunoglobulins”), and references cited therein.

Hybridoma cell lines that produce monoclonal antibodies specific for the target molecules are contemplated herein. Such hybridomas can be produced and identified by conventional techniques. For the production of antibodies, various host animals may be immunized by injection the polypeptide of interest or antigenic fragments thereof. Such host animals may include, but are not limited to, horse, goat, sheep, cow, rabbits, mice, and rats, to name a few. Various adjuvants may be used to increase the immunological response. Depending on the host species, such adjuvants include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. The monoclonal antibodies can be recovered by conventional techniques. Such monoclonal antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the mAb may be cultivated in vitro or in vivo. Or, the antibody genes can be cloned and optionally otherwise altered, and expressed in another cell line approved for recombinant production of protein pharmaceuticals such as, for example, CHO cells.

Alternatively, libraries of antibody fragments can be screened and used to develop human antibodies through recombinant techniques. Such libraries are commercially available from, for example, Cambridge Antibody Technology (Melbourne, UK), and Morphosys (Munich, Del.). In addition, techniques developed for the production of “chimeric antibodies” (Takeda et al., *Nature*, 314:452, 1985) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used.

A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a porcine mAb and a human immunoglobulin constant region. The monoclonal antibodies of the invention also include humanized versions of murine monoclonal antibodies. Such humanized antibodies can be prepared by known techniques and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. For example, transgenic mice into which genetic material encoding one or more human immunoglobulin chains has been introduced may be employed. Such mice may be genetically altered in a variety of ways. The genetic manipulation may result in human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains in at least some (preferably virtually all) antibodies produced by the animal upon immunization.

Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (Nature 332:323, 1988), Liu et al. (PNAS 84:3439, 1987), Larrick et al. (Bio/Technology 7:934, 1989), and Winter and Harris (TIPS 14:139, Can, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Pat. Nos. 5,569,825 and 5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein. For use in humans, the antibodies are typically human or humanized; techniques for creating such human antibodies are also known. Transgenic animals for making human antibodies are available from, for example, Medarex Inc. (Princeton, N.J.) Protein Design Labs, Inc. (Fremont, Calif.) and Abgenix Inc. (Fremont, Calif.).

Expression of a humanized immunoglobulin sequences in bacterial hosts may be used to select higher affinity humanized immunoglobulin sequences by mutagenizing the CDR regions and producing bacteriophage display libraries which may be screened for humanized immunoglobulin CDR variants which possess high affinity and/or high specificity binding to the target molecules. One potential advantage of such affinity sharpening is the generation of humanized immunoglobulin CDR variants that have improved binding affinity and/or reduced cross-reactivity with molecules other than the molecule to which they were raised. Methods for producing phage display libraries having immunoglobulin variable region sequences are provided in the art (see, e.g., Cesareni, FEBS Lett 307:66, 1992; Swimmer et al., Proc. Natl. Acad. Sci. USA 89:3756, 1992; Gram et al., Proc. Natl. Acad. Sci. USA 89:3576, 1992; Clackson et al., Nature 352:624, 1991; Scott & Smith, Science 249:386, 1990; Garrard et al., Bio/Techniques 9:1373, 1991; which are incorporated herein by reference in their entirety for all

purposes. The resultant affinity sharpened CDR variant humanized immunoglobulin sequences are subsequently expressed in a suitable host.

Antibody fragments, which recognize specific epitopes, may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the (ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., Science, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. Techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, Science 242:423, 1988; Huston et al., Proc. Natl. Acad. Sci. USA 85:5879, 1988; and Ward et al., Nature 334:544, 1989) can also be adapted to produce single chain antibodies against polypeptides of interest. In addition, such antibodies can, in turn, be utilized to generate anti-idiotypic antibodies using techniques known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J 7(5):437, 1993; and Nissinoff, J. Immunol 147(8):2429, 1991).

Nucleic Acid-Based IL-18BP antagonists

In alternative embodiments, nucleic acid-based immunotherapy can be designed to decrease the level of endogenous IL-18BP gene expression, e.g., using antisense or ribozyme approaches to inhibit or prevent translation of IL18BP mRNA transcripts; triple helix approaches to inhibit transcription of IL18BP; or targeted homologous recombination to inactivate or “knock out” the IL18BP gene or its endogenous promoter.

Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing polypeptide translation. Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to a target mRNA of interest having a complementary polynucleotide sequence. Absolute complementarity, although preferred, is not required. A sequence “complementary” to a portion of RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, thereby forming a stable duplex. Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of the targeted gene

transcript could be used in an antisense approach to inhibit translation of endogenous target proteins. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, and the like. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. W088/09810, published Dec. 15, 1988), or hybridization-triggered cleavage agents or intercalating agents (see, e.g., Zon, 1988, Pharm. Res. 5:539-549).

The antisense molecules are delivered to cells, which express a transcript having an IL18BP polynucleotide sequence in vivo by, for example, injecting directly into the tissue or cell derivation site, or by use of modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically or to host cells obtained from the subject to be treated. Another approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the subject will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous IL18BP transcripts and thereby prevent translation of the targeted mRNA. For example, a vector can be introduced in vivo such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, so long as it can be transcribed to produce the desired antisense RNA. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells.

Ribozyme molecules designed to catalytically cleave mRNA transcripts having an IL18BP polynucleotide sequence prevent translation of the target mRNA (see, e.g., PCT International Publication WO90/11364, published Oct. 4, 1990; U.S. Pat. No. 5,824,519).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated. There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, Nature, 334:585-591, 1988) and “hammerhead”-type. Tetrahymena-type ribozymes recognize sequences, which are four bases in length, while “hammerhead”-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved stability, targeting, and the like). A typical method of delivery involves using a DNA construct “encoding” the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous autoinflammatory mRNA and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Alternatively, endogenous IL-18BP expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (i.e., the target gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target gene (see generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Antisense RNA and DNA, ribozyme, and triple helix molecules described herein may be prepared by any method known in the art for the synthesis of DNA and RNA molecules and include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides such as, for example, solid phase phosphoramidite chemical synthesis, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, and the like). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al., 1988, Nucl. Acids Res. 16:3209. Methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451). Alternatively, RNA molecules may be

generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters.

Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or
5 inducibly, depending on the promoter used, can be introduced stably into cell lines.

In alternative embodiments IL-18BP expression may be blocked by post-translational gene silencing, such as by double-stranded RNA-induced gene silencing, also known as RNA interference (RNAi). RNA sequences specific for IL-18BP may be modified to provide double-stranded sequences or short hairpin RNAs for therapeutic use.

10

Screening for IL-18, IL-18R, and DR-18 Agonists

IL-18, IL-18R and DR-18 agonists can be evaluated using screening assays known in the art, such as high throughput test systems. The assays can be performed in a variety of formats, including protein-protein binding assays, competition binding assays, biochemical screening
15 assays, immunoassays, cell-based assays, etc. For the sake of clarity, the following exemplary assays are described in the context of IL-18, IL-18R, DR-18 and are therefore illustrative and not limiting.

By observing the effect that an IL-18 agonist has on the interaction between IL-18 and IL-18 receptor in various binding assays, on IL-18/IL-18 receptor-mediated activity in functional
20 tests, and in cell based screens, molecules that are potential therapeutics can be identified because they augment the interaction between IL-18 and IL-18 receptor and enhance the immune-protective functions of IL-18. IL-18 agonists that partially or completely enhance IL-18 binding to IL-18 receptor, and hence the activation related to anti-inflammatory activities of IL-18 receptor, can be useful as immunosuppressants or anti-inflammatory agents in to ameliorate
25 symptoms resulting from pathogenic, autoreactive CD4+ T cells.

In another aspect, the screening methods entail incubation of the test compound, the IL-18 protein and cells expressing IL-18 receptor for a suitable time period; determining the level of biological activity of IL-18 on the IL-18 receptor in presence of the test compound; and
30 comparing the level of biological activity with that which occurs in the absence of test compound, wherein a difference in the level of biological activity indicates that the test compound affects the biological activity of the IL-18/IL-18 receptor complex. Biological activity

of IL-18 on the IL-18 receptor can be assayed in any number of ways, for example but not limited to, determining the phosphorylation state of intracellular proteins (i.e., activation of the IL-18 receptor by IL-18); determining the production of proinflammatory factors, such as Interferon gamma (IFN γ), IL-6, IL-8, monocyte chemoattractant protein-1 and Groa;

5 determining the production of hematopoietic cytokines, such as G-CSF and GM-CSF and IL-8; determining increased expression of IL-1 β and TNF, as well as measuring induction of iNOS in macrophages, and determining any immune protective factors produced.

A particular example of an assay for the identification of potential IL-18 agonists is a competitive assay, which combines IL-18 and an IL-18 receptor-specific agonist with IL-
10 receptor under the appropriate conditions for a competitive assay. Either IL-18 or the IL-18 receptor-specific agonist can be labeled so that the binding can be determined, and the effectiveness of the agonist judged. The label allows for detection by direct or indirect means. Direct means include, but are not limited to luminescence, radioactivity, optical or electron density. Indirect means include but are not limited to an enzyme or epitope tag.

15 Another method by which IL-18 agonists can be identified that inhibit the interaction between IL-18 and IL-18 receptor is the solid phase method, in which IL-18 receptor is bound and placed in a medium with labeled IL-18. The amount of signal produced by the interaction between IL-18 and IL-18 receptor is measured in the presence and in the absence of a test compound. Diminished levels of signal, in comparison to a control, indicate that the test
20 compound inhibited the interaction between IL-18 and IL-18 receptor. Increased levels of signal, in comparison to a control, indicate that the candidate molecule promotes the interaction between IL-18 and IL-18 receptor. In alternative embodiments, IL-18 could be bound and IL-18 receptor labeled. The IL-18, IL-18 receptor agonists and/or IL-18BP antagonists can be directly or indirectly labeled. For example, if the protein is recombinantly produced, one can engineer
25 fusion proteins that can facilitate solubility, labeling, immobilization and/or detection. Fusion proteins which facilitate these processes can include, but are not limited to soluble Ig-tailed fusion proteins and His-tagged proteins. Methods for engineering such soluble Ig-tailed fusion proteins are well known to those of skill in the art. See, for example, U.S. Pat. No. 5,116,964, and the illustrative embodiments described below. Indirect labeling involves the use of a protein,
30 such as a labeled antibody, which specifically binds to a component of the assay. IL-18, IL-18R and DR-18 agonists can be identified and evaluated using cells and/or cell lines derived from

isolated neuronal cells, splenocytes, lymph node cells, immune cells, T cells, B cells and spinal cord cells and cell lines may be used to evaluate IL-18, IL-18R and DR-18 agonists or IL-18BP antagonists in any of the suitable assays described herein. Biologically relevant readouts in the cell-based assay may be used to evaluate potential modulators, such as cell survival;

- 5 hypertrophic responses; and/or production of molecules in response to hypoxic, environmental stress or immunopathology.

IL-18 modulators can also be identified using methods that are well suited for high-throughput screening procedures, such as scintillation proximity assays (Udenfriend et al., 1985, Proc Natl Acad Sci USA 82: 8672-8676), yeast two-hybrid or interaction trap assays,
10 homogeneous time-resolved fluorescence methods (Park et al., 1999, Anal Biochem 269: 94-104), fluorescence resonance energy transfer (FRET) methods (Clegg R M, 1995, Curr Opin Biotechnol 6: 103-110), or methods that measure any changes in surface plasmon resonance when a bound polypeptide is exposed to a potential binding partner, using for example a biosensor such as that supplied by Biacore AB (Uppsala, Sweden).

15 Compounds that can be assayed that may also be IL-18, and IL-18R agonists include but are not limited to small organic molecules, such as those that are commercially available - often as part of large combinatorial chemistry compound 'libraries'—from companies such as Sigma-Aldrich (St. Louis, Mo.), Arqule (Woburn, Mass.), EnzyMed (Iowa City, Iowa), Maybridge Chemical Co.(Trevillet, Cornwall, UK), MDS Panlabs (Bothell, Wash.), Pharmacopeia
20 (Princeton, N.J.), and Trega (San Diego, Calif.). Preferred small organic molecules for screening using these assays are usually less than 10K molecular weight and can possess a number of physicochemical and pharmacological properties which enhance cell penetration, resist degradation, and/or prolong their physiological half-lives (Gibbs, J., 1994, Pharmaceutical Research in Molecular Oncology, Cell 79(2): 193-198). Compounds including natural products,
25 inorganic chemicals, and biologically active materials such as proteins and toxins can also be assayed using these methods to identify molecules having IL-18, IL-18R, an DR-18 protective immune cell activity. In turn these molecules can be manipulated by IL-18 agonists or mimetics to enhance these activities in target cells. For example, IL-18, IL-18R and DR-18 agonists can be administered to a cell or group of cells to increase IL-18:IL-18R, binding and thereby increase
30 therapeutically beneficial cellular communication, cell stimulation, or activity in the target cells. In such an assay, one would determine a rate of communication or cell stimulation in the

presence of the IL-18:IL-18R binding and then determine if such communication or cell stimulation is altered in the presence of the agonist. Exemplary assays for this aspect of the invention include cytokine secretion assays, T-cell co-stimulation assays, and mixed lymphocyte reactions involving antigen presenting cells and T cells. These assays are well known to those skilled in the art.

IL-18 agonists may regulate cytokine, cell proliferation (either inducing or inhibiting), or cell differentiation (either inducing or inhibiting) activity, or may induce production of other cytokines in certain cell populations. Many polypeptide factors discovered to date have exhibited such activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cell stimulatory activity. The activity of the agonists of interest may be evidenced by any one of a number of factor-dependent cell proliferation assays for cell lines including, without limitation, NF κ B, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RBS, DA1, 123, T1165, HT2, CTLL2, TF-1, Mole and CMK.

The activity of IL-18: IL-18R binding may, among other means, be measured by the following methods:

Assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of cellular adhesion under static conditions 7.28.1-7.28.22), Takai et al., PNAS USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (pp. 3.1-3.19: In vitro assays for mouse lymphocyte function; Chapter 7. Immunologic studies in humans); Takai et al., J. Immunol. 137: 3494-3500, 1986; Bertagnolli et al., J. Immunol. 145: 1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Kruisbeek and Shevach, 1994, Polyclonal T cell stimulation, in Current Protocols in Immunology, Coligan et al. eds. Vol 1 pp.

3.12.1-3.12.14, John Wiley and Sons, Toronto; and Schreiber, 1994, Measurement of mouse and human interferon gamma in Current Protocols in Immunology, Coligan et al. eds. Vol 1 pp.

6.8.1-6.8.8, John Wiley and Sons, Toronto.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Bottomly et al., 1991, Measurement of human and murine interleukin 2 and interleukin 4, in Current Protocols in Immunology, Coligan et al. eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto; deVries et al., J Exp Med 173: 1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc Natl Acad Sci.USA 80: 2931-2938, 1983; Nordan, 1991, Measurement of mouse and human interleukin 6, in Current Protocols in Immunology Coligan et al. eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto; Smith et al., Proc Natl Acad Sci USA 83: 1857-1861, 1986; Bennett et al., 1991, Measurement of human interleukin 11, in Current Protocols in Immunology Coligan et al. eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto; Ciarletta et al., 1991, Measurement of mouse and human Interleukin 9, in Current Protocols in Immunology; Coligan et al. eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto.

Assays for T-cell clone responses to antigens (which will identify, among others, polypeptides that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3: In vitro assays for mouse lymphocyte function; Chapter 6: Cytokines and their cellular receptors; Chapter 7: Immunologic studies in humans); Weinberger et al., PNAS USA 77: 6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988

Assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., PNAS USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., PNAS USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-

3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, polypeptides that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J Immunol 144: 3028-3033, 1990; and Mond and Brunswick, 1994, Assays for B cell function: in vitro antibody production, in Current Protocols in Immunology Coligan et al. eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, polypeptides that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol 137:3494-3500, 1986; Takai et al., J. Immunol 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, polypeptides expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol 134:536-544, 1995; Inaba et al., J Exp Med 173:549-559, 1991; Macatonia et al., J Immunol 154:5071-5079, 1995; Porgador et al., J Exp Med 182:255-260, 1995; Nair et al., J Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., J Exp Med 169:1255-1264, 1989; Bhardwaj et al., J Clin Invest 94:797-807, 1994; and Inaba et al., J Exp Med 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, polypeptides that prevent apoptosis after superantigen induction and polypeptides that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, J Immunol 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for polypeptides that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cell Immunol 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., PNAS USA 88:7548-7551, 1991.

5 Assays for embryonic stem cell differentiation (which will identify, among others, polypeptides that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

 Assays for cell movement and adhesion include, without limitation, those described in:
10 Current Protocols in Immunology Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta chemokines 6.12.1-6.12.28); Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J Immunol. 152:5860-5867, 1994; Johnston et al. J Immunol. 153: 1762-1768, 1994

15 Assays for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

20 **Therapeutic Compositions and Administration Thereof**

 This disclosure provides compounds, compositions, and methods for treating a subject, preferably a human patient, who is suffering from immunopathology resulting from pathogenic or autoreactive CD4+ T cell disease symptoms. The terms “treat”, “treating”, and “treatment” used herein includes curative, preventative (e.g., prophylactic) and palliative or ameliorative
25 treatment. Modulating therapeutic compositions of IL-18 agonist or mimetics may be administered before, during, and/or after the presentation of symptoms. For therapeutic use, a soluble IL-18, IL-18R and DR-18 modulator is administered to a subject for treatment in a manner appropriate to the indication.

 Embodiments of the present disclosure include therapeutic compositions (also referred to
30 as pharmaceutical compositions) comprising one or more soluble IL-18, IL-18R, DR-18 agonists and/or one or more IL-18BP antagonist. A “therapeutic composition,” as used herein, comprises

one or more soluble IL-18, IL-18R, DR-18, or IL-18BP modulators and a pharmaceutically acceptable diluent, preservative, solubilizer, emulsifier, adjuvant and/or carrier. As used herein, the terms “pharmaceutically” acceptable and “physiologically” acceptable are used interchangeably. The term “pharmaceutically acceptable” means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

Embodiments include therapeutic compositions (also referred to as pharmaceutical compositions) comprising one or more soluble IL-18, IL-18R, DR-18 agonists. A “therapeutic composition,” as used herein, comprises one or more soluble IL-18, IL-18R, and DR-18 modulators and a pharmaceutically acceptable diluent, preservative, solubilizer, emulsifier, adjuvant and/or carrier. As used herein, the terms “pharmaceutically” acceptable and “physiologically” acceptable are used interchangeably. The term “pharmaceutically acceptable” means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

Therefore, in certain aspects, the therapeutic compositions comprise one or more of the modulators described in the sections above: e.g., soluble receptor molecules, as well as biologically active fragments, muteins, variants, derivatives, fusions, etc. thereof; antibodies, fusion proteins and/or peptibodies directed against one or more of the following: IL-18, IL-18R, DR-18, IL-18BP; small molecules, such as peptidomimetics, mimotopes and the like, that enhance the interaction between IL-18 and IL-18R; antisense oligonucleotides that specifically target and hybridize to the mRNA of endogenous IL-18BP to inhibit or prevent translation of IL-18BP mRNA transcripts; and RNA-interference molecules tailored to silence expression of IL-18BP. Preferred peptide mimetics comprising active fragments of the proteins listed above that can inhibit or augment the activities of the protein. Peptide mimetics can be 10, 20, 20, 40 or 50 amino acids in length and can optionally be modified to increase bioavailability upon administration to a subject.

Physiologically acceptable carriers, excipients or diluents are nontoxic to recipients at the dosages and concentrations employed. Ordinarily, preparing such compositions entails combining the modulators of interest with buffers, antioxidants such as ascorbic acid, low molecular weight polypeptides (such as those having fewer than 10 amino acids), proteins, amino acids, carbohydrates such as glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with

conspecific serum albumin are exemplary appropriate diluents. The agonists and antagonists preferably are formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents in certain embodiments. Appropriate dosages can be determined in standard dosing trials, and may vary according to the chosen route of administration. In accordance with
5 appropriate industry standards, preservatives may also be added, such as benzyl alcohol. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the age and condition of the patient, and so forth.

In one embodiment, sustained-release forms of soluble IL-18, IL-18R, DR-18, agonists or
10 IL-18BP antagonists described herein, are used. Sustained-release forms suitable for use in the disclosed methods include but are not limited to, use of molecules that are encapsulated in a slowly dissolving biocompatible polymer, admixed with such a polymer, and or encased in a biocompatible semi-permeable implant. In addition, the modulator molecules may be conjugated with polyethylene glycol (pegylated) to prolong its serum half-life or to enhance protein delivery
15 (as described in detail above).

One type of sustained release technology that may be used in administering soluble target modulator therapeutic compositions is that utilizing hydrogel materials, for example, photopolymerizable hydrogels (Sawhney et al., *Macromolecules* 26:581; 1993). Similar hydrogels have been used to prevent postsurgical adhesion formation (Hill-West et al., *Obstet. Gynecol.* 83:59, 1994) and to prevent thrombosis and vessel narrowing following vascular injury
20 (Hill-West et al., *Proc. Natl. Acad. Sci. USA* 91:5967, 1994). Polypeptides can be incorporated into such hydrogels to provide sustained, localized release of active agents (West and Hubbel, *Reactive Polymers* 25:139, 1995; Hill-West et al., *J. Surg. Res.* 58:759; 1995). The sustained, localized release of IL-18 agonist or mimetic for example, when incorporated into hydrogels
25 would be amplified by the long half-life of IL-18.

Therapeutic compositions may be for administration for injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, the invention encompasses therapeutic compositions comprising effective amounts one or more of the agonists and antagonists of the invention individually or together with pharmaceutically acceptable diluents,
30 preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength;

additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference in their entirety. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

Contemplated for use herein are oral solid dosage forms, which are described generally in Chapter 89 of Remington's Pharmaceutical Sciences (1990), 18th Ed., Mack Publishing Co. Easton Pa. 18042, which is herein incorporated by reference in its entirety. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Pat. No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Pat. No. 5,013,556).

A description of possible solid dosage forms for the therapeutic is given in Chapter 10 of Marshall, K., Modern Pharmaceutics (1979), edited by G. S. Banker and C. T. Rhodes, herein incorporated by reference in its entirety. In general, the formulation will include one or more IL-18, IL-18R, DR-18, and IL-18BP modulators and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

Also specifically contemplated are oral dosage forms of the above inventive compounds. If necessary, the compounds may be chemically modified so that oral delivery is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the compound molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the compound and increase in circulation time in the body. Moieties useful as covalently attached vehicles in this invention may also be used for this purpose. Examples of

such moieties include: PEG, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. See, for example, Abuchowski and Davis, Soluble Polymer-Enzyme Adducts, Enzymes as Drugs (1981), Hocenberg and Roberts, eds., Wiley-Interscience, New York, N.Y., pp. 367-83; Newmark, et al. (1982), J. Appl. Biochem. 4:185-9. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are PEG moieties.

For oral delivery dosage forms, it is also possible to use a salt of a modified aliphatic amino acid, such as sodium N-(8-[2-hydroxybenzoyl]amino)caprylate (SNAC), as a carrier to enhance absorption of the therapeutic compounds of this invention. The clinical efficacy of a heparin formulation using SNAC has been demonstrated in a Phase II trial conducted by Emisphere Technologies. See U.S. Pat. No. 5,792,451, "Oral drug delivery composition and methods".

The compounds of this invention can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the compound of the invention with an inert material. These diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell. Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrants include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the

disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An anti-frictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the compound of this invention into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives may also be included in the formulation to enhance uptake of the compound. Additives potentially having this property are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

Controlled release formulation may be desirable. The compound of this invention could be incorporated into an inert matrix which permits release by either diffusion or leaching

mechanisms; e.g., gums. Slowly degenerating matrices may also be incorporated into the formulation, e.g., alginates, polysaccharides. Another form of a controlled release of the compounds of this invention is by a method based on the Oros therapeutic system (Alza Corp.), i.e., the drug is enclosed in a semipermeable membrane which allows water to enter and push
5 drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

Other coatings may be used for the formulation. These include a variety of sugars which could be applied in a coating pan. The therapeutic agent could also be given in a film coated tablet and the materials used in this instance are divided into 2 groups. The first are the
10 nonenteric materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

A mix of materials might be used to provide the optimum film coating. Film coating may
15 be carried out in a pan coater or in a fluidized bed or by compression coating.

Also contemplated herein is pulmonary delivery of the present protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei
20 et al., *Pharma. Res.* (1990) 7: 565-9; Adjei et al. (1990), *Internatl. J. Pharmaceutics* 63: 135-44 (leuprolide acetate); Braquet et al. (1989), *J. Cardiovasc. Pharmacol.* 13 (suppl.5): s.143-146 (endothelin-1); Hubbard et al. (1989), *Annals Int. Med.* 3: 206-12 (α 1-antitrypsin); Smith et al. (1989), *J. Clin. Invest.* 84: 1145-6 (α 1-proteinase); Oswein et al. (March 1990), "Aerosolization of Proteins", *Proc. Symp. Resp. Drug Delivery II*, Keystone, Colo. (recombinant human growth hormone); Debs et al. (1988), *J. Immunol.* 140: 3482-8 (interferon- γ and tumor necrosis factor α)
25 and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor).

In certain approaches, a wide range of mechanical devices designed for pulmonary delivery of therapeutic products are employed, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this invention
30 are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Mo.; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colo.; the Ventolin metered

dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, N.C.; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass.

All such devices require the use of formulations suitable for the dispensing of the inventive compound. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to diluents, adjuvants and/or carriers useful in therapy.

The inventive compound should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

Nasal delivery of the inventive compounds is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic compound to the nose, without the necessity for deposition of the product in the lung.

Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucous membranes is also contemplated.

One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature and severity of the disorder to be treated, the patient's body weight, age, general condition, and prior illnesses and/or treatments, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices such as standard dosing trials. For example, the therapeutically effective dose can be estimated initially from cell culture assays. The dosage will depend on the specific activity of the compound and can be readily determined by routine experimentation. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture, while minimizing toxicities. Such information can be used to more accurately determine useful doses in humans. Ultimately, the attending physician will decide the amount of polypeptide of the present invention with which to treat each individual patient.

Initially, the attending physician will administer low doses of the inventive compounds of the present invention and observe the patient's response. Larger doses of the compounds of the

present invention can be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various therapeutic compositions used to practice the method of the present invention should contain about 0.01 ng to about 100 mg (preferably about 0.1 ng to about 10 mg, more preferably about 0.1 microgram to about 1 mg) of polypeptide of the present invention per kg body weight. In one embodiment of the invention, the inventive therapeutic compounds are administered one time per week to treat the various medical disorders disclosed herein, in another embodiment is administered at least two times per week, and in another embodiment is administered at least three times per week. If injected, the effective amount of therapeutic compounds per adult dose ranges from 1-20 mg/m², and preferably is about 5-12 mg/m². Alternatively, a flat dose can be administered, whose amount may range from 5-100 mg/dose. Exemplary dose ranges for a flat dose to be administered by subcutaneous injection are 5-25 mg/dose, 25-50 mg/dose and 50-100 mg/dose. In one embodiment of the invention, the various indications described below are treated by administering a preparation acceptable for injection containing the compounds at 25 mg/dose, or alternatively, containing 50 mg per dose. The 25 mg or 50 mg dose can be administered repeatedly, particularly for chronic conditions. If a route of administration other than injection is used, the dose is appropriately adjusted in accord with standard medical practices. In many instances, an improvement in a patient's condition will be obtained by injecting a dose of about 25 mg one to three times per week over a period of at least three weeks, or a dose of 50 mg one or two times per week for at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For incurable chronic conditions, the regimen can be continued indefinitely, with adjustments being made to dose and frequency if such are deemed necessary by the patient's physician. The foregoing doses are examples for an adult patient who is a person who is 18 years of age or older. For pediatric patients (age 4-17), a suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of the therapeutic compounds administered by subcutaneous injection one or more times per week. If the compound is in the form of an antibody, a preferred dose range is 0.1 to 20 mg/kg, and more preferably is 1-10 mg/kg.

Another embodiment of a dose range is 0.75 to 7.5 mg/kg of body weight. Humanized antibodies are preferred, that is, antibodies in which only the antigen-binding portion of the

antibody molecule is derived from a non-human source. Such antibodies can be injected or administered intravenously.

“Concurrent administration” encompasses simultaneous or sequential treatment with the components of the combination, as well as regimens in which the drugs are alternated, or
5 wherein one component is administered long-term and the other(s) are administered intermittently. Components can be administered in the same or in separate compositions, and by the same or different routes of administration.

Examples of other drugs or therapeutic compositions that may be used in combination with inventive compounds of the invention alone or in combination include: analgesic agents,
10 disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and any immune and/or inflammatory modulators. Non-steroidal anti-inflammatories may include, but are not limited to: salicylic acid (aspirin); ibuprofen; indomethacin; celecoxib; rituxin, rofecoxib; ketorolac; nambumetone; piroxicam; naproxen; oxaprozin; sulindac; ketoprofen; diclofenac; other COX-1 and/or COX-2 inhibitors, salicylic acid derivatives,
15 propionic acid derivatives, acetic acid derivatives, fumaric acid derivatives, carboxylic acid derivatives, butyric acid derivatives, oxicams, pyrazoles and pyrazolones, including newly developed anti-inflammatories.

Therapeutic compositions of this invention may be administered with one or more of the following: modulators of other members of the TNF/TNF receptor family, including TNF
20 antagonists, such as etanercept (Enbrel™), sTNF-RI, onercept, D2E7, and Remicade™; IL-1 inhibitors, including IL-1ra molecules such as anakinra and more recently discovered IL-1ra-like molecules such as IL-1Hy1 and IL-1Hy2; IL-1 “trap” molecules as described in U.S. Pat. No. 5,844,099; IL-1 antibodies; solubilized IL-1 receptor, and the like; IL-6 inhibitors (e.g., antibodies to IL-6); IL-8 inhibitors (e.g., antibodies to IL-8); Interleukin-1 converting enzyme
25 (ICE) modulators; insulin-like growth factors (IGF-1, IGF-2) and modulators thereof; transforming growth factor- β (TGF- β), TGF- β family members, and TGF- β modulators; fibroblast growth factors FGF-1 to FGF-10, and FGF modulators; COX-2 inhibitors, such as Celebrex™ and Vioxx™; prostaglandin analogs (e.g., E series prostaglandins); matrix metalloproteinase (MMP) modulators; nitric oxide synthase (NOS) modulators, including
30 modulators of inducible NOS; modulators of glucocorticoid receptor; modulators of glutamate

receptor; modulators of lipopolysaccharide (LPS) levels; anti-cancer agents, including inhibitors of oncogenes (e.g., fos, jun) and interferons; noradrenaline and modulators and mimetics thereof.

Additional embodiments of compositions that can be administered concurrently with the pharmaceutical compositions of the invention are: cytokines, lymphokines, or other

5 hematopoietic factors such as M-CSF, GM-CSF, Flt3-Ligand, TNF, IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin, or inhibitors or antagonists of any of these factors. The pharmaceutical composition can further contain other agents which either enhance the activity of the polypeptide or complement its activity or use in treatment. Such
10 additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with polypeptide of the invention, or to minimize side effects. Conversely, the inventive compounds may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or
15 anti-thrombotic factor, or anti-inflammatory agent.

Further embodiments of drugs to be administered concurrently include but are not limited to antivirals, antibiotics, analgesics, corticosteroids, antagonists of inflammatory cytokines, non-steroidal anti-inflammatories, pentoxifylline, thalidomide, and disease-modifying antirheumatic drugs (DMARDs) such as azathioprine, cyclophosphamide, cyclosporine, hydroxychloroquine
20 sulfate, methotrexate, leflunomide, minocycline, penicillamine, sulfasalazine and gold compounds such as oral gold, gold sodium thiomalate, and aurothioglucose.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed,
25 various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. The invention having been described, the following examples are offered by way of illustration, and not limitation.

30 The following materials and methods are provided to facilitate the practice of the present disclosure.

Mice:

All mice were kept in a specific-pathogen free facility and all experiments performed under a protocol approved by the Children's Hospital of Philadelphia Institutional Animal Care and Use Committee. WT and C57BL/6N-*Il18bp*^{tm1.1(KOMP)VLcg} (*Il18bp*^{-/-}) mice were obtained via the Knock-out Mouse Project (KOMP, UC San Diego <https://mmrrc.ucdavis.edu/mta/KOMP-MTA.pdf>). IL-18 transgenic mice (*Il18tg*) were a gift from T. Hoshino, Kurume University¹⁸. 2D2 mice were purchased from the Jackson Laboratory. All 2D2 mice were screened using flow cytometric analysis of PBMCs using specific antibodies to Vα3.2 and Vβ11 (ThermoFisher Scientific). All mice used in experiments were males, 6-9 weeks of age at the initiation of the studies. All animal procedures were performed in accordance with approved university protocols.

Experimental Autoimmune Encephalomyelitis (EAE):

EAE was induced according to a previously established protocol³¹. Briefly, mice were immunized subcutaneously in four sites on the back with a total of 200 µg MOG (35–55) peptide (Biosynthesis) emulsified with CFA with *M. tuberculosis* strain H37Ra (DIFCO Laboratories). Mice were injected with 200 ng pertussis toxin (List Biological Laboratories) intraperitoneally on days 0 and 2. Mice were assessed daily and scored as follows: 1, flaccid tail; 2, impaired righting reflex and hindlimb weakness; 3, complete hindlimb paralysis; 4, hindlimb paralysis with partial forelimb paralysis; or 5, moribund.

rm-IL-18 Treatment:

WT mice were administered recombinant murine IL-18 (MBL, Nagoya, Japan, 0.2µg/injection as in Kinoshita et al.³²) or PBS in three intraperitoneal injections (i.p.) during the early induction phase of EAE (at days 0, 2, and 4) and during the late effector phase of EAE (at days 9, 11, and 13).

Flow Cytometry:

Single-cell suspensions were obtained from spleen, lymph node, and spinal cord tissues by mechanical isolation adapted from previously published protocols^{33, 34}. For spinal cord immune cells, mice were euthanized, perfused with saline, and spinal cords were removed using

hydraulic extrusion. Spinal cords were minced using surgical scissors and digested for 30 minutes in a 37°C incubator in a Collagenase D/DNAse I mixture. Single cell suspensions were then homogenized using an 18G needle and passed through a 70µM filter. Immune cells were then obtained using a Percoll gradient (30%/60%) centrifugation and collected from the interphase. Cells were washed and stained with antibodies conjugated to fluorochrome dyes. Flow cytometry was performed on a Beckman Coulter CytoFLEX LX and analyzed using FlowJo.

Antibodies used for this analysis include Zombie UV (BioLegend), Tcrb in PE-Cy7 (Tonbo Biosciences), CD8 in AF700 (BD), CD4 in APC Cy-7 (Invitrogen), IFNγ in BV488 (BD), IL-17a in PerCP Cy 5.5 (Invitrogen), RORγT in PE (Invitrogen), T-bet in BV421 (Invitrogen), FOXP3 in FITC (Invitrogen), and FC Block (BioLegend).

The following examples are provided to illustrate certain embodiments of the subject matter described. They are not intended to limit the invention in any way.

Example I

We sought to determine whether or not free or chronic excess of IL-18 would exacerbate or ameliorate the clinical phenotypes of an EAE model. Subcutaneous immunization with 200µg of MOG₃₅₋₅₅ and two injections of 100 ng of pertussis toxin on days 0 and 2 in mice that do not express IL-18BP, the decoy receptor for IL-18, (i.e., IL-18BP knockout mice, *Il18bp*^{-/-}) and mice that overexpress IL-18 (i.e., mice carrying an IL-18 transgene, *Il18tg*¹⁸) resulted in little to no clinical paralysis in these mice (Fig.1B and Fig. 1C). To elucidate the importance of timing on the effect of IL-18 in this context, we sought to block IL-18 signaling at either EAE initiation or after establishment of disease. We proceeded to inject wildtype B6 mice with recombinant mouse IL-18 (rmIL-18) at the induction phase (Days 0, 2, and 4), or during the effector phase (Days 9, 11, 13). Interestingly, we found that early addition of rmIL-18 resulted in a delay of paralysis (Fig.1D). We hypothesized that inhibition of rmIL-18 by endogenous IL-18BP would prevent the effects of rmIL-18 administration in the early phase. To more fully address the effects of exogenous IL-18 signaling, we treated WT mice with either PBS or mouse IL-18BP “decoy resistant” IL-18 (also known as mouse “DR-18”; see e.g., Zhou et al.¹⁴) and found strong protection from EAE with mouse DR-18 administration (Fig. 1E) without signs of hyperinflammation. To better evaluate the impact of timing of IL-18 signaling on inhibiting

EAE, we administered a IL-18 receptor blocking antibody to wildtype and *Il18bp*^{-/-} mice at various timepoints, including early in disease, throughout the experiment, or later in the course. Early and continuous IL-18 signaling blockade resulted in loss of protection, but so too did blocking IL-18 signaling after the onset of clinical symptoms (Fig. 1F).

5 Through our experiments, to our surprise, we observed a relative increase in CD8 T-cells in the spinal cords of *Il18bp*^{-/-} mice or mice administered DR-18, but not control mice without excess IL-18 (Fig. 2A and Fig. 2B). This relative increase was largely due to a diminution of conventional, but not FoxP3⁺, CD4 T-cells. 2D2 transgenic mice, which harbor a transgene encoding an MHCII-restricted T-cell Receptor (TCR) specific for a MOG₃₅₋₅₅ peptide, have very
10 few CD8 T cells due to allelic exclusion, but a great abundance of autoreactive, MOG-specific CD4 T cells. We then sought to determine whether or not crossing these 2D2 transgenic mice to *Il18bp*^{-/-} mice would make *Il18bp*^{-/-} susceptible to clinical paralysis in EAE. Surprisingly, the loss of CD8 T cells and expansion of MOG specific CD4 T cells abrogated the protection of *Il18bp*^{-/-} mice from EAE (Fig. 2C).

15 Having shown the beneficial effects of IL-18 in EAE in a variety of systems, and its dependence on the IL-18 receptor, to better characterize what cell types were responding to IL-18 and thereby initiating the protective response we used several transgenic mice. *Il18tg* mice specifically lacking the *Il18r1* gene in T-cells were no longer protected from EAE, and the proportion of the spinal cord cells that were CD8⁺ was no longer elevated, showing that both
20 protection and CD8 T-cell abundance in the CNS required T-cell IL-18 signaling (Fig. 3A-B). This protection could have been mediated by FoxP3⁺ Treg responses to IL-18, but deletion of *Il18r1* specifically from these cells did not affect protection in *Il18tg* mice (Fig. 3C). Finally, to further refine the IL-18 responsive cell type, we induced EAE in *Il18tg* mice who, upon tamoxifen treatment, deleted the *Il18r1* gene only in mature CD8 T-cells. The temporal loss of
25 *Il18r1* only in mature CD8 T-cells was sufficient to abrogate the protective effect of IL-18 in EAE, proving that CD8 T-cell responses to IL-18 were required. Though spinal cord CD8 T-cells were equally activated (CD44⁺) in mice treated with PBS or DR-18, the latter showed greater expression of Helios and less Ly49 (Fig. 4A-B). Ly49 expression has been proposed as a marker of CD8Treg – although high expression of Ly49 may impair CD8Treg function¹². Helios is a
30 transcription factor typically associated with CD4Treg, but in models of autoimmunity Helios may be important for CD8Treg function³⁵.

To better understand the role of IL-18 on CD8Treg, we recovered splenic CD8 T-cells from WT EAE-immunized mice at day 10 post-immunization, cultured splenocytes ex vivo with or without IL-18 for 3 days, and then purified CD8 T-cells for adoptive transfer into newly-immunized WT mice (Fig. 5A). Transfer of IL-18-conditioned CD8 T-cells delayed the onset of EAE by a few days, but did not result in profound protection (Fig. 5B). This suggested the IL-18-conditioned CD8Treg may be more efficacious, but likely needed ongoing IL-18 stimulation. CD8Treg are postulated to protect from autoimmunity by acting specifically on autoreactive CD4 T-cells (CD4Tauto). In preparation for determining the fate of transferred 2D2 CD4Tauto, we found that it took a remarkably high number of transferred 2D2 cells to overcome protection in *Il18bp-/-* mice. Interestingly, whereas transfer for 1.25 to 6 million 2D2 cells overcame protection, transfer of 250,000 2D2 cells enhanced the already impressive protection in *Il18bp-/-* mice (Fig. 5C). One interpretation of this result is that low numbers of 2D2 cells transferred at the time of EAE initiation provided an important antigen presenting cell specifically to IL-18-hungry CD8Tregs.

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While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. It will be apparent to one skilled in the art that various changes and modifications
30 can be made therein without departing from the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. A method for ameliorating disease activity in autoimmune, allergic, or post-transplant disorders in a subject in need thereof, comprising; administering to said subject an effective amount of an agent that increases the immune protective effects of Interleukin-18 (IL-18) to promote one or more of differentiation, expansion, and function of therapeutically beneficial IL-18 responsive lymphocytes, thereby suppressing immunopathology caused by pathogenic lymphocytes in said subject, and improving inflammatory symptoms.
2. A method for treating an autoimmune disease caused by a harmful pro-inflammatory response to a self-antigen in a subject in need thereof, comprising; administering to a subject having the self-antigen, an interleukin-18 receptor (IL-18R) agonist in an amount effective to ameliorate a symptom of the autoimmune disease, thereby improving autoimmune symptoms mediated in said subject.
3. A method for treating an inflammatory disease in a subject, comprising administering to said subject an effective amount of an agent that enhances the immune protective effects of Interleukin-18 (IL-18) indirectly via effects on antigen presenting cell function, thereby improving an inflammatory disease mediated by an indirectly-acting APC signal.
4. A method for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof, comprising
administering to said subject an effective amount of a therapeutic population of cells comprising one or more of CD8+ T-cells, Natural Killer (NK) cells, CD4+ regulatory T-cells, cultured or conditioned in the presence of an agonist that mimics or enhances the immune protective effects of IL-18; optionally the method further comprising contacting said one or more of CD8+ T-cells, Natural Killer (NK) cells, CD4+ regulatory T-cells with said agonist; thereby suppressing immunopathology resulting from the pathogenic CD4+ T cells present in said subject.
5. A method for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof, comprising administering to said subject an effective amount of an antagonist that inhibits activity of IL-18 Binding Protein (IL-18BP), thereby increasing at least one of bioactivity and enhancement of immune protective effects of free IL-18 in said subject,

thereby suppressing immunopathology resulting from the pathogenic CD4+ T cells and alleviating inflammatory symptoms in said subject.

6. The method of any one of claims 1-5, wherein said one or more lymphocyte populations is selected from a suppressive CD8+ T cell, Natural Killer (NK) cell, NKT cell, and CD4+ regulatory T-cells.

7. The method of any one of claims 1-6, wherein the disease or disorder is selected from the group consisting of: multiple sclerosis, rheumatoid arthritis, myasthenia gravis, Addison's disease, celiac disease, type 1 diabetes mellitus, autoimmune thyroiditis, ulcerative colitis, Sjogren syndrome, systemic lupus erythematosus, Grave's disease, Crohn's disease, Waldenstrom's macroglobulinemia, hyperviscosity syndrome, monoclonal gammopathy of undetermined origin, POEMS syndrome, myeloma, and macroglobulinemia.

8. The method of claim 1, or claim 3, or claim 5, wherein said CD4+ cells are autoreactive.

9. The method of any one of claims 1 to claim 6, wherein said allergic disorder is selected from atopic dermatitis, allergic rhinosinusitis, allergic asthma, and eosinophilic esophagitis

10. The method of any one of claims 1 to 6, wherein said post-transplant disorder is graft versus host disease (GvHD).

11. The method of any one of claims 1 to 10, wherein bioactivity of free IL-18 is increased in said subject.

12. The method of any one of claims 1 to 11, further comprising administration of at least one additional agent, wherein the at least one additional agent comprises one or more of IL-18, IL-18 receptor agonists and IL-18BP antagonist.

13. The method of claim 11 or 12, wherein bioactivity of free IL-18 is increased and IL-18BP binding is reduced.

14. The method of any one of claims 1 to 13, wherein an IL-18 receptor agonists that is an IL-18 mimetic is administered.

15. The method of any one of claims 1 to 14, wherein said agent, said IL-18R agonist, or said agonist is decoy resistant IL-18 (DR-18).

16. The method of claim 15, wherein the DR-18 comprises an amino acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with at least one of SEQ ID NOs: 2-63.
- 5 17. The method of claim 16, wherein the DR-18 comprises one or more mutations at positions Y1, L5, K8, C38, M51, K53, S55, Q56, P57, G59, M60, C68, E77, Q103, S105, D110, N111, M113, V153, and N155 relative to the wild-type IL-18 amino acid sequence set forth in SEQ ID NO:108.
- 10 18. The method of claim 17, wherein the DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 20, 7, 17-19, 21, 22, 55-57, 62 and 63.
19. The method of any one of claims 15 to 18, wherein the amount of the DR-18 administered is from 15 µg/kg to 3000 µg/kg.
20. The method of any one of the previous claims, wherein said agent, agonist or antagonist is administered via a route selected from systemic, intravenous, or subcutaneous administration.
- 15 21. The method of claim 6, wherein the differentiation, expansion, and, or function of suppressive CD8+ T cells induced by said agent is monitored.
22. The method of claim 3, wherein signaling from said APC engages MHC class II molecules which interact with T cell receptors, said APC being selected from dendritic cells, macrophages, B cells, thymic epithelial cells, vascular endothelial cells, and follicular dendritic cells.
- 20 23. The method of any of the previous claims, wherein said immune protective effects of free IL-18 include one or more of
- i) binding of IL-18 to the IL-18R and activation of the IL-18R;
 - ii) regulation of innate and acquired immune responses;
 - iii) induction of one or more T-lymphocyte helper (Th1) responses;
 - 25 iv) enhanced cell-mediated cytotoxicity;
 - v) IFN-γ induction;

- vi) enhanced production of GM-CSF and IL-2;
- vii) potentiation of anti-CD3 induced T-cell proliferation;
- viii) increased Fas-mediated killing by natural killer cells (NK cells) and CD4+ Th1 cells;
- 5 ix) increased apoptotic death via activation of the Fas-FasL pathway signaling;
- x) up-regulation of FasL expression;
- xi) induction of T-lymphocyte helper cell type 2 responses (Th2) in T-cells and NK cells;
- xii) stimulation of basophils and mast cells to produce Th2 cytokines and histamine;
- 10 xiii) inhibition of IgE production; and
- xiv) activation of T cell signaling pathways by APC cells.

24. The method of claim 5, wherein said antagonist is selected from an inhibitory nucleic acid, an inhibitory antibody, a peptide mimetic, a small molecule, or an IL-18 decoy-to-the-decoy (D2D).

- 15 25. The method of claim 24, wherein the IL-18 D2D comprises an amino acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with at least one of SEQ ID NOs: 64-97.

- 20 26. The method of claim 25, wherein the DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 64-97.

27. The method of any one of the previous claims, further comprising administration of one or more anti-inflammatory agents selected from analgesic agents, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs).

- 25 28. The method of claim 27, further comprising administration of one or more antiviral, antibiotic, analgesic, corticosteroid, antagonists of inflammatory cytokine, a non-steroidal anti-inflammatory agent, pentoxifylline, thalidomide, azathioprine, cyclophosphamide, cyclosporine, hydroxychloroquine sulfate, methotrexate, leflunomide, minocycline, penicillamine,

sulfasalazine, indomethacin; celecoxib; rituxin, rofecoxib; ketorolac; nambumetone; piroxicam; naproxen; oxaprozin; sulindac; ketoprofen; diclofenac; and gold compounds such as oral gold, gold sodium thiomalate, and aurothioglucose.

29. The method of claim 4, wherein said culturing or said conditions produce Suppressive
5 CD122+CD8+ T-cells and/or KIR+CD8+ T-cells.

30. The method of any one of the previous claims, wherein said cells are donor cells haplotype-matched to the subject to be treated.

31. The method of any one of the previous claims wherein said cells are autologous and harvested from the subject to be treated.

10 32. Use of a composition comprising an agonist of interleukin 18 (IL-18) or IL-18 receptor (IL-18R), in a method of treating a subject, according to any one of the preceding claims, wherein the subject is a human subject having a disease or disorder selected from the group consisting of: autoimmune disease or disorder, allergic disease or disorder, post-transplant disease or disorder, and an inflammatory disease caused by pathogenic CD4+ T cells; and the method treats the
15 subject for the disease or disorder.

33. The use according to claim 32, wherein the disease or disorder is multiple sclerosis.

34. The use according to claim 32 or 33, wherein the agonist is a decoy resistant IL-18 (DR-18).

35. The use according to claim 34, wherein the DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 20, 7, 17-19, 21, 22, 55-57, 62 and 63.

20 36. Use of a composition comprising an antagonist of interleukin 18 binding protein (IL-18BP), in a method of treating a subject, according to any one of the preceding claims, wherein the subject is a human subject having a disease or disorder selected from the group consisting of: autoimmune disease or disorder, allergic disease or disorder, post-transplant disease or disorder, and an inflammatory disease caused by pathogenic CD4+ T cells; and the method treats the
25 subject for the disease or disorder.

37. The use according to claim 36, wherein the disease or disorder is multiple sclerosis.

38. The use according to claim 36 or 37, wherein the antagonist is an interleukin 18 decoy-to-the-decoy (IL-18 D2D).

39. The use according to claim 36 or 37, wherein the antagonist is an IL-18BP inhibitory antibody that specifically binds IL-18BP.

5 40. The use according to claim 36 or 37, wherein the antagonist is an IL-18BP inhibitory nucleic acid that prevents translation and/or expression of a nucleic acid encoding IL-18BP.

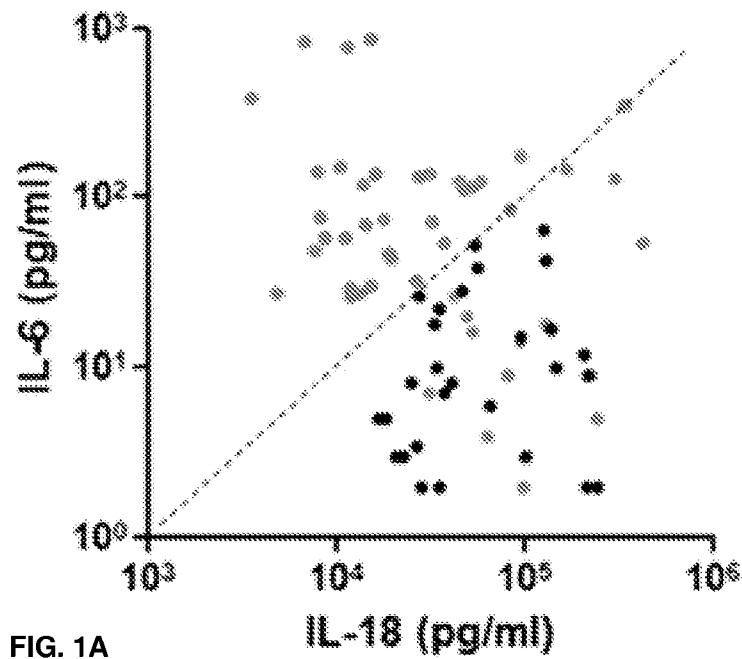


FIG. 1A

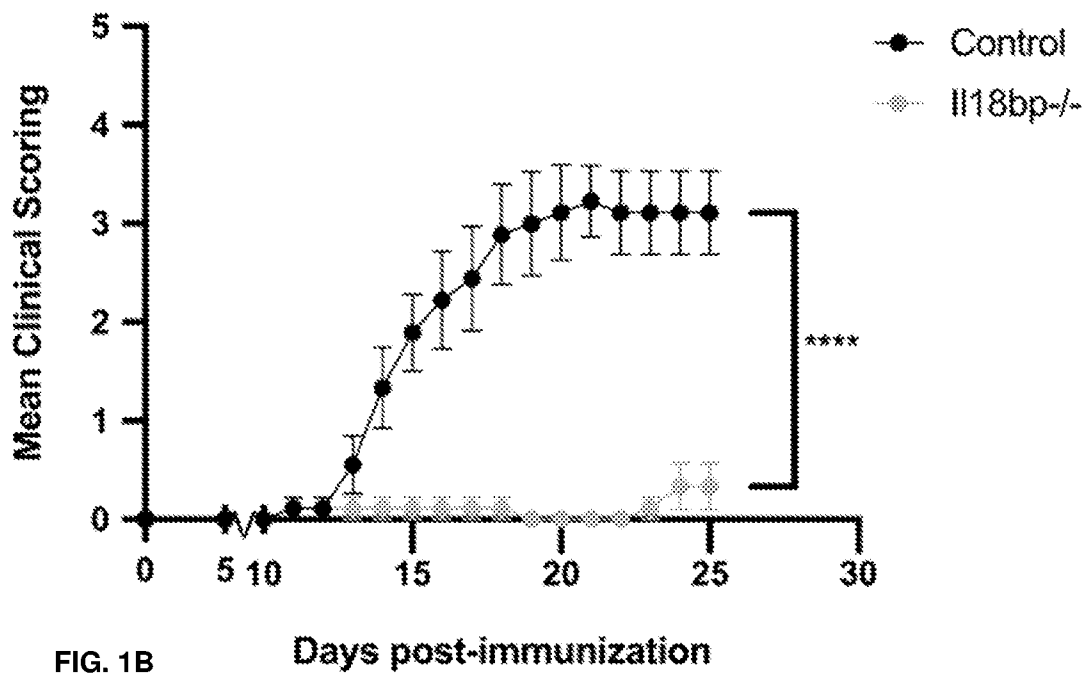


FIG. 1B

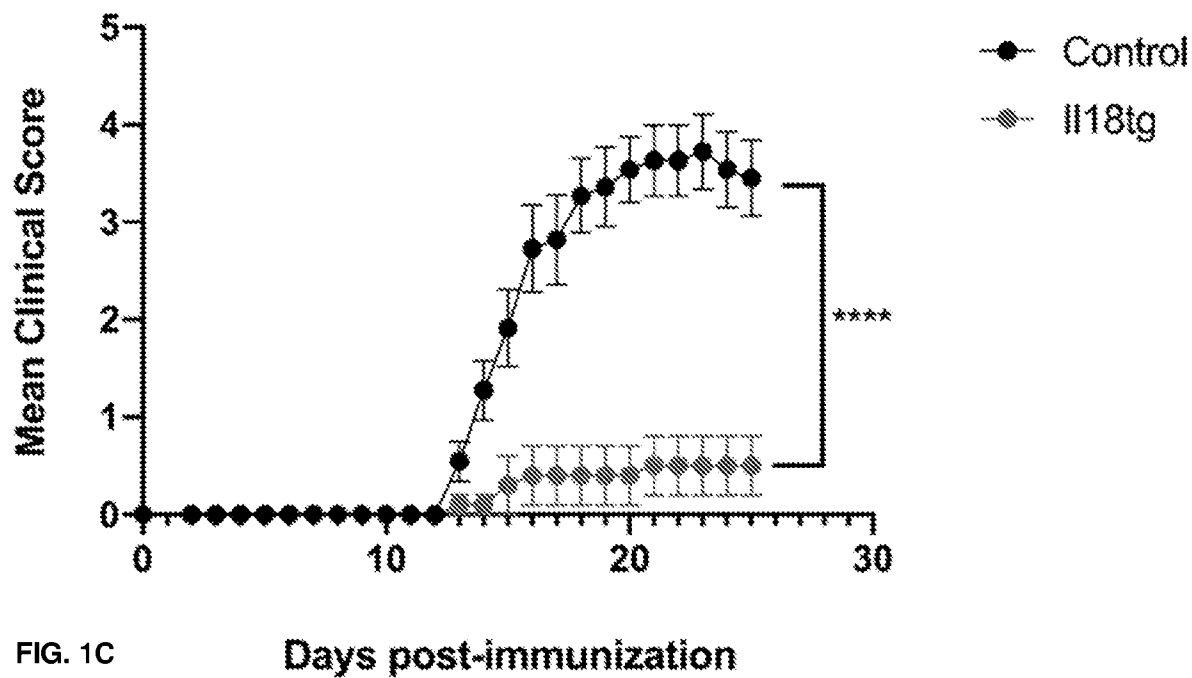


FIG. 1C

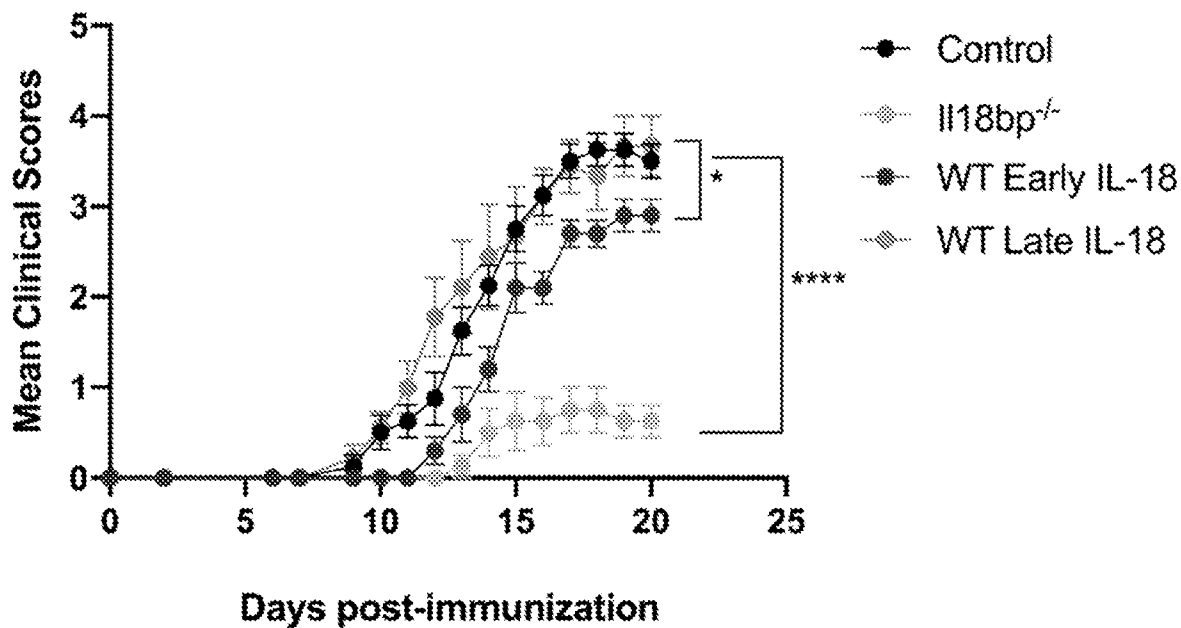


FIG. 1D

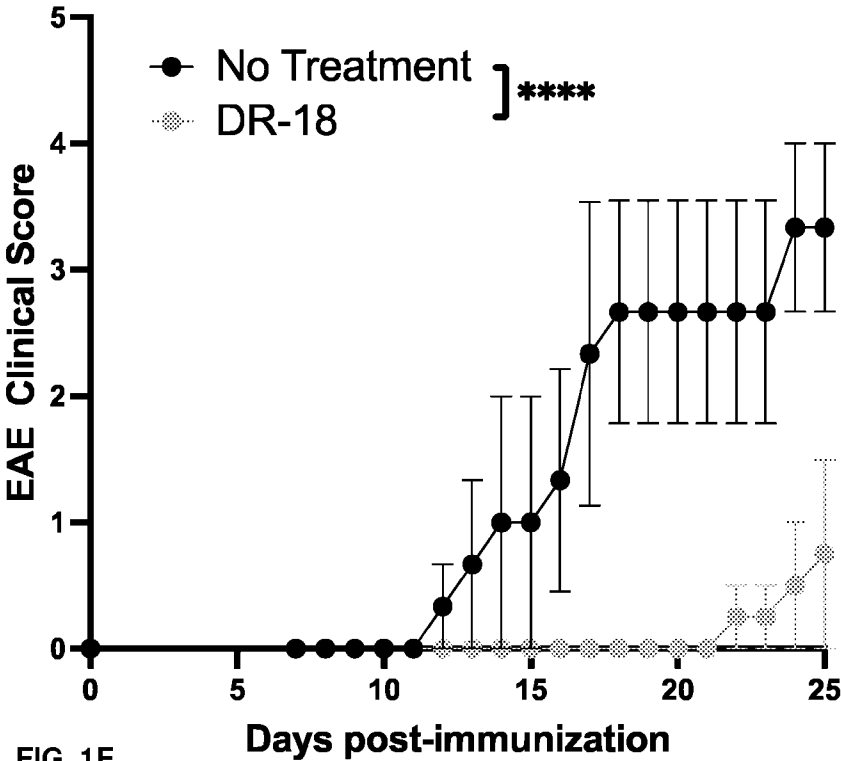


FIG. 1E

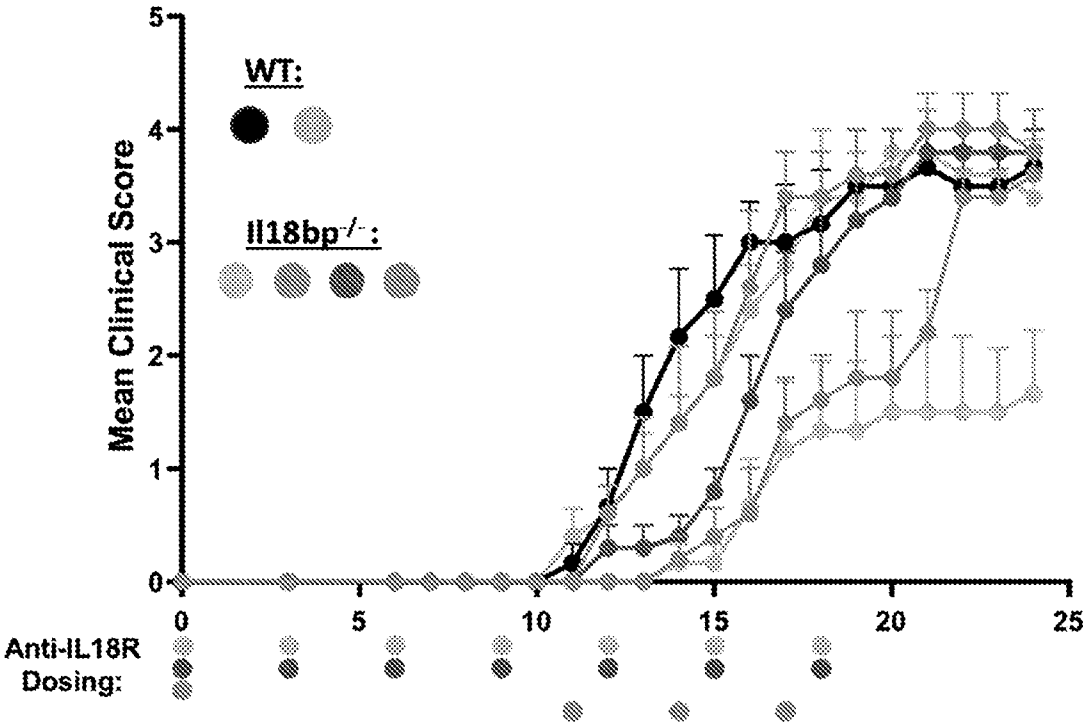


FIG. 1F

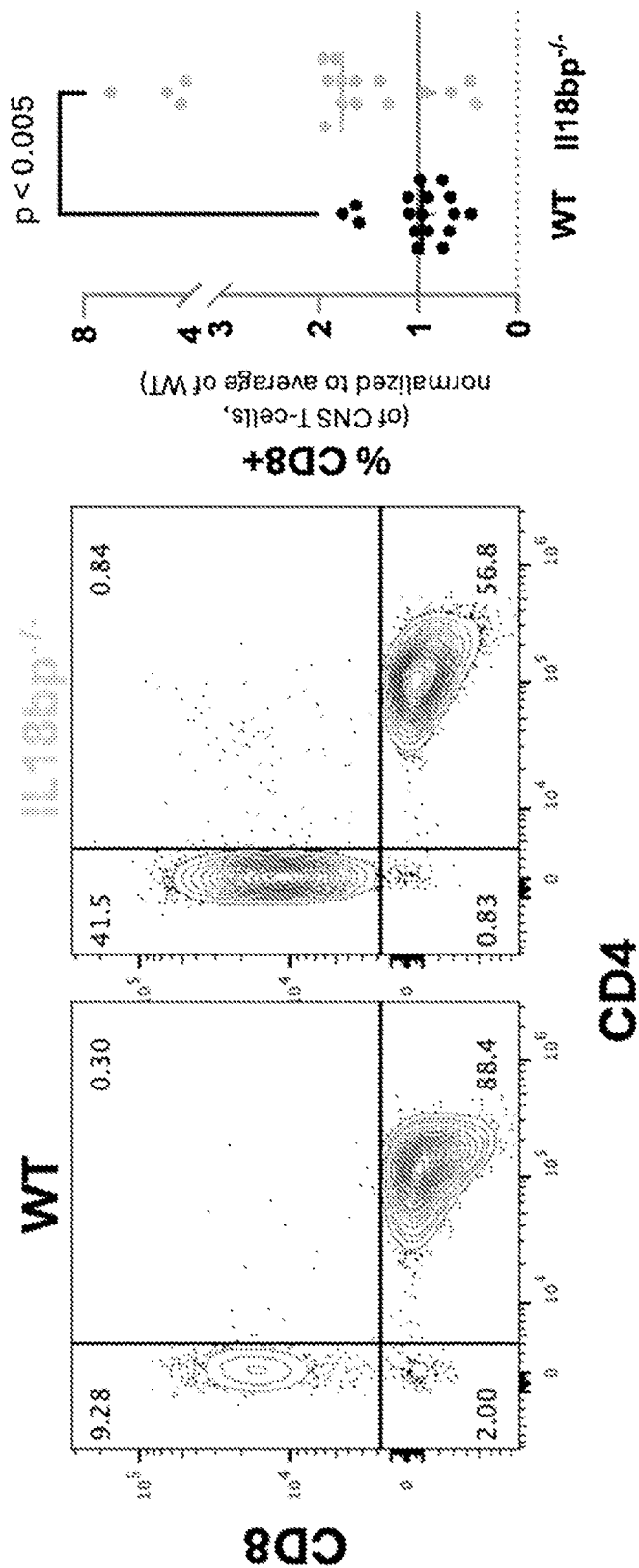


FIG. 2A

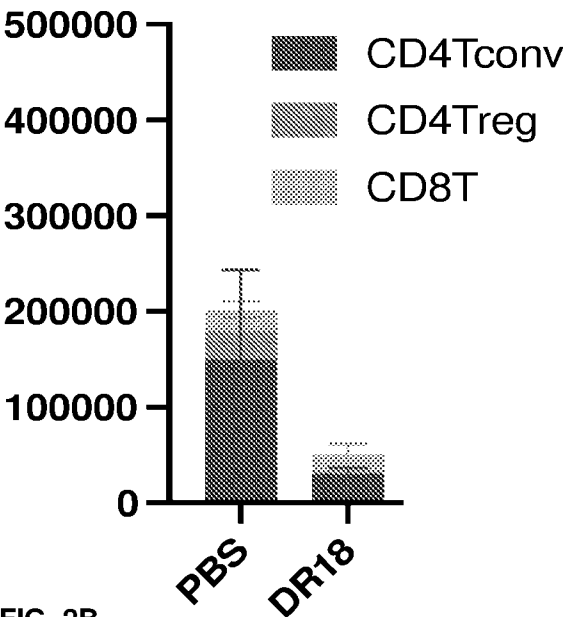


FIG. 2B

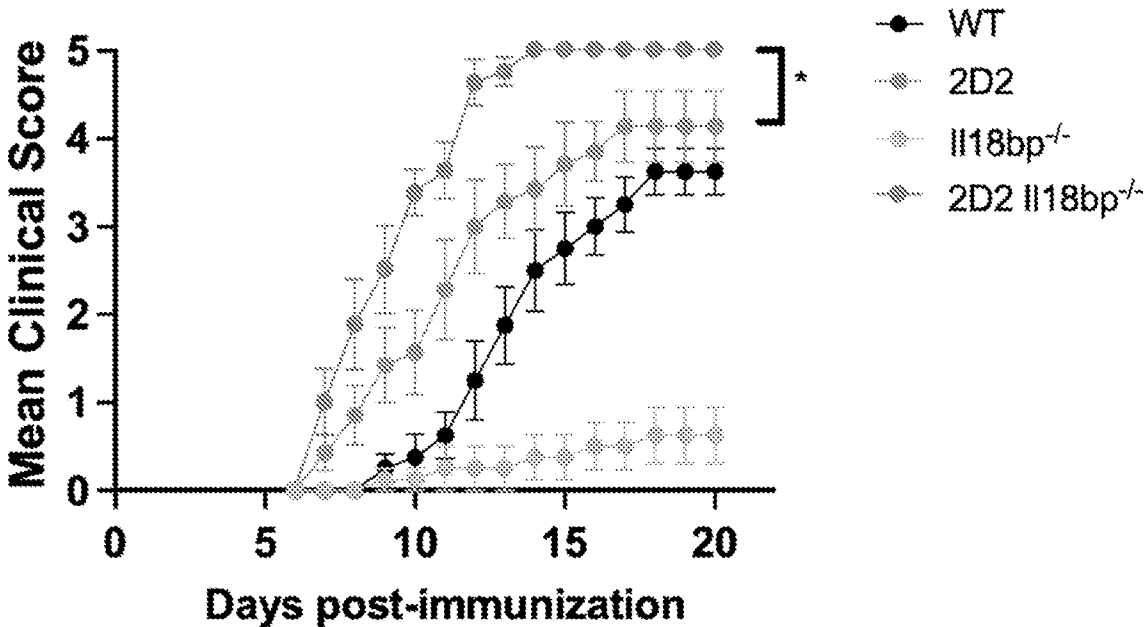


FIG. 2C

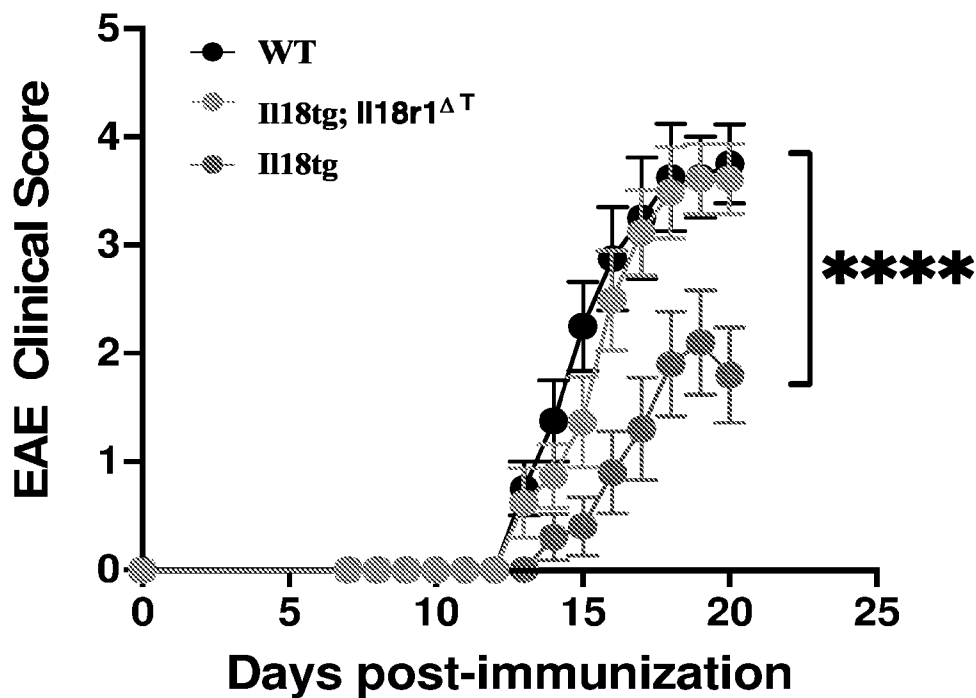


FIG. 3A

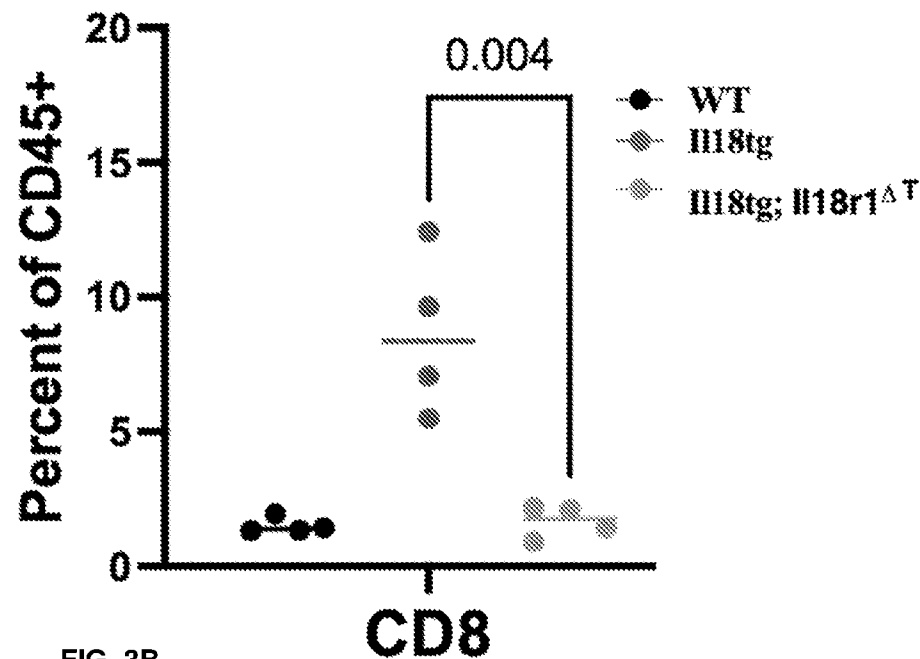


FIG. 3B

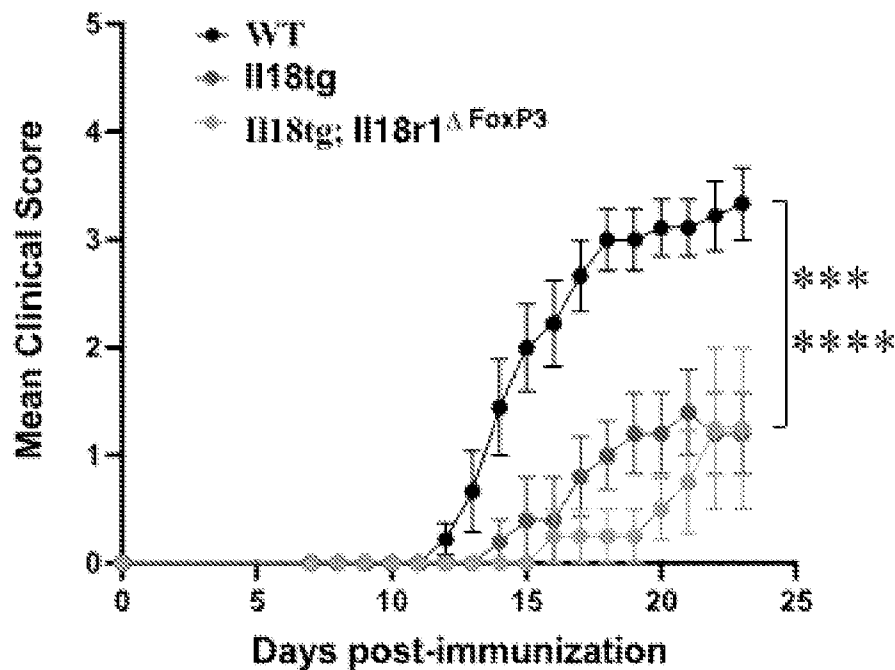


FIG. 3C

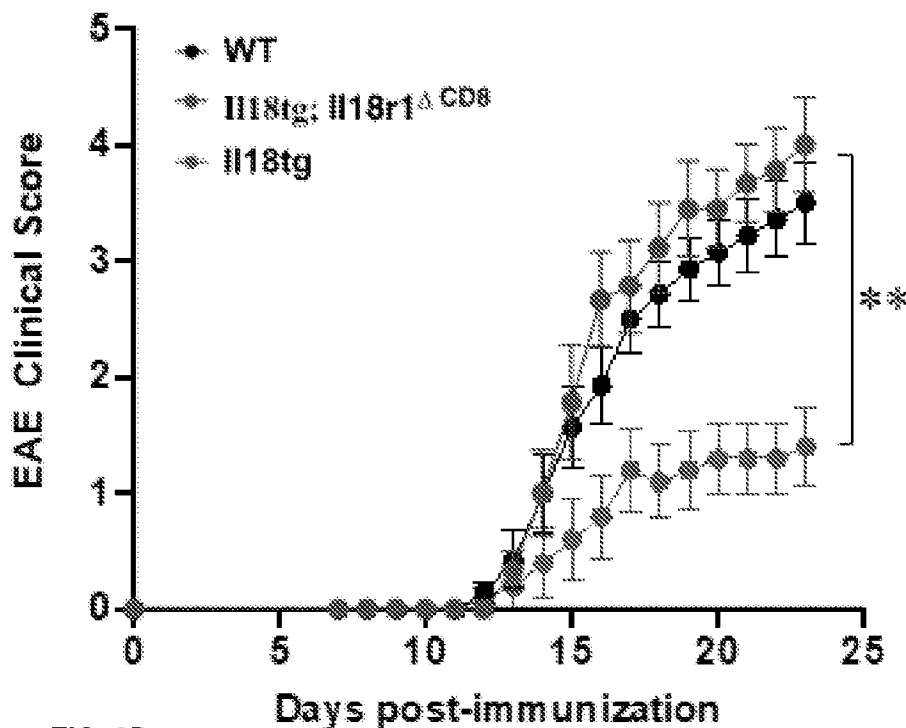


FIG. 3D

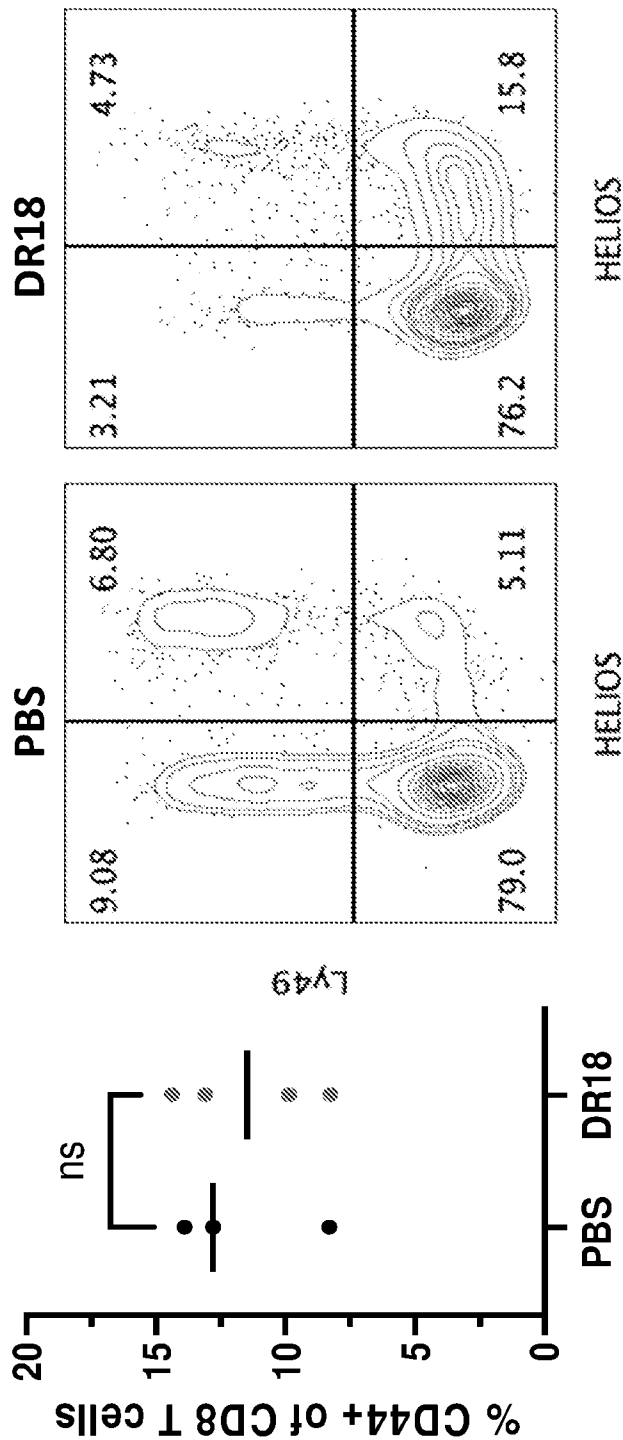


FIG. 4A

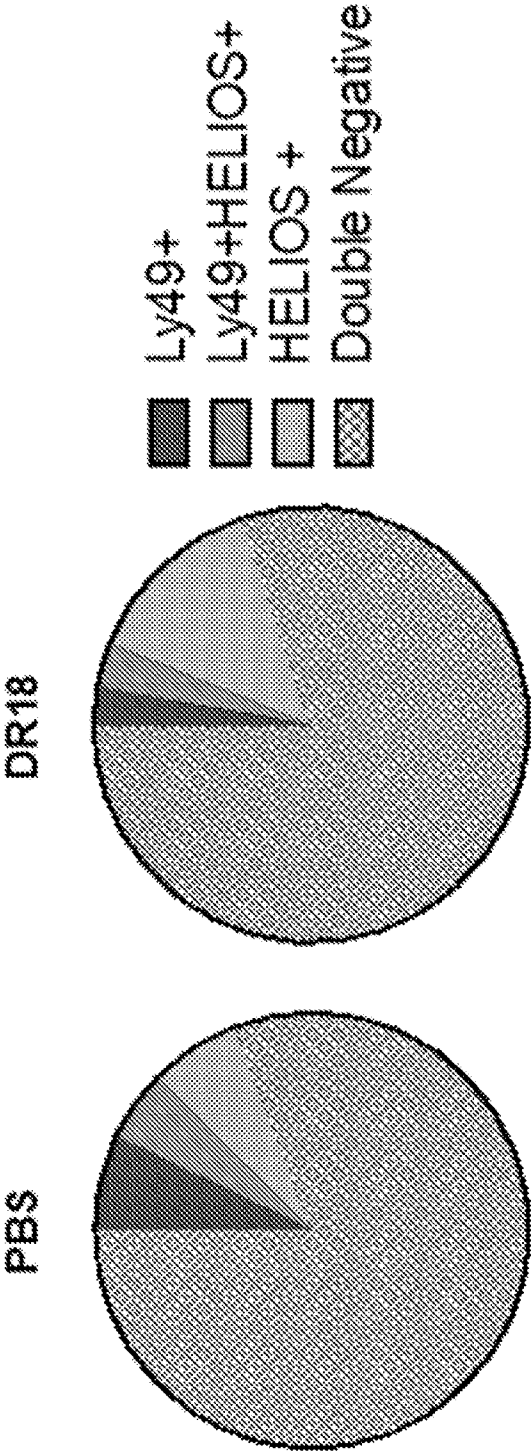


FIG. 4B

10/11

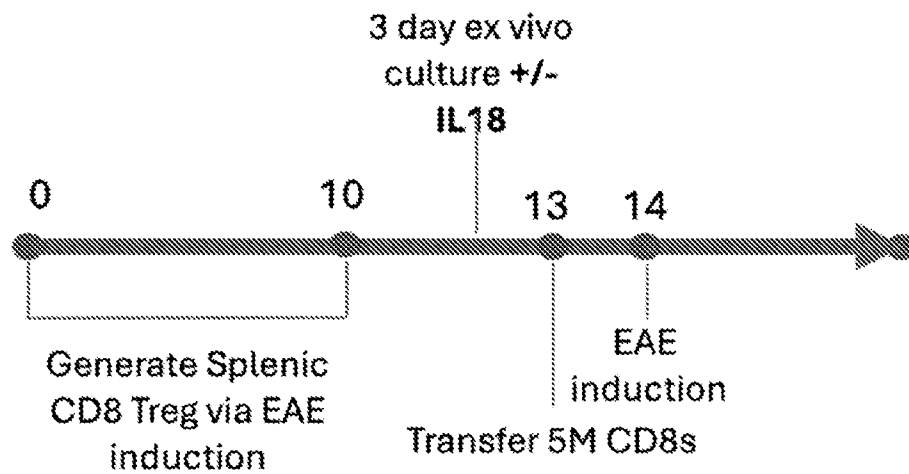


FIG. 5A

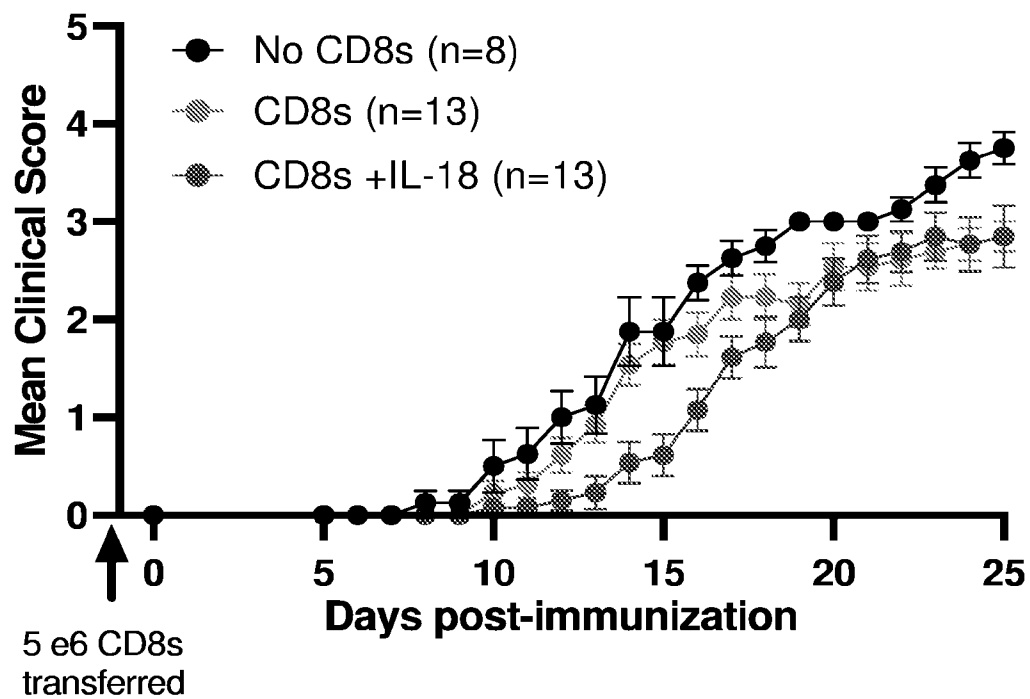


FIG. 5B

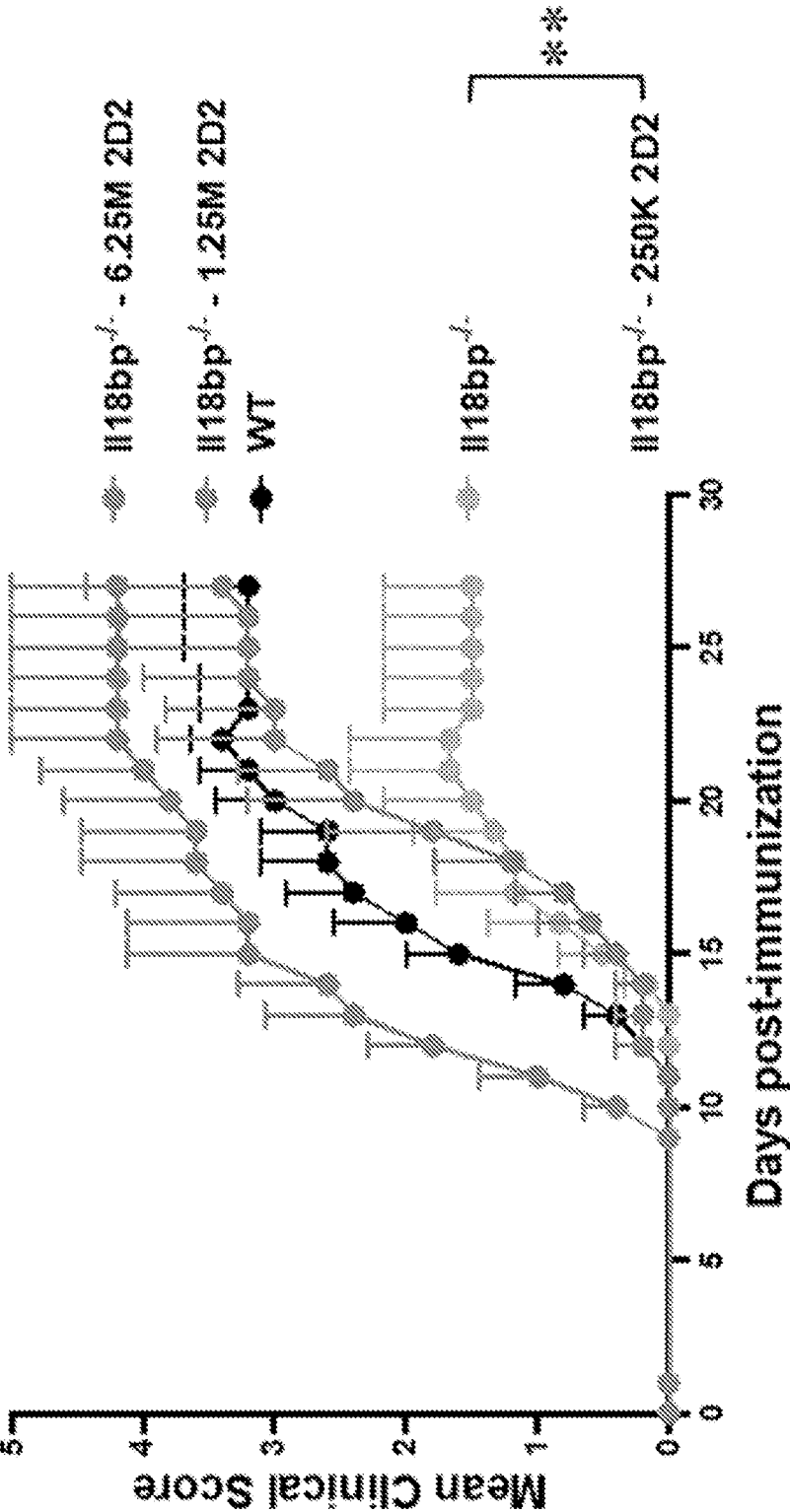


FIG. 5C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/037468

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/20** (2024.01); **A61P 29/00** (2024.01); **A61P 37/06** (2024.01); **C07K 14/54** (2024.01); A61K 35/17 (2024.01)
CPC: **A61K 38/20**; **A61P 29/00**; **A61P 37/06**; **C07K 14/54**; A61K 35/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2001/0044418 A1 (LEVY et al.) 22 November 2001 (22.11.2001)	
Y	entire document	1, 6
Y	entire document	21
Y	US 2020/0297837 A1 (21C BIO) 24 September 2020 (24.09.2020)	
Y	entire document	21
A	US 2002/0068057 A1 (FELDMANN et al.) 06 June 2002 (06.06.2002)	
A	entire document	1, 6, 21
A	US 2012/0263764 A1 (WATSON) 18 October 2012 (18.10.2012)	
A	entire document	1, 6, 21
A	US 2019/0070262 A1 (YALE UNIVERSITY) 07 March 2019 (07.03.2019)	
A	entire document	1, 6, 21

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

30 August 2024 (30.08.2024)

Date of mailing of the international search report

24 October 2024 (24.10.2024)

Name and mailing address of the ISA/US

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Facsimile No. **571-273-8300**

Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/037468

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **7, 9-20, 23, 27, 28, 30-40**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claim 1, and also claims 6 and 21 (in part) are drawn to methods for ameliorating disease activity in autoimmune, allergic, or post-transplant disorders in a subject in need thereof.

Group II: claim 2, and also claims 6 and 21 (in part) are drawn to methods for treating an autoimmune disease caused by a harmful pro-inflammatory response to a self-antigen in a subject in need thereof.

Group III: claims 3 and 22, and also claims 6 and 21 (in part) are drawn to methods for treating an inflammatory disease in a subject.

Group IV: claims 4 and 29, and also claims 6 and 21 (in part) are drawn to methods for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof comprising administering to said subject an effective amount of a therapeutic population of cells.

Group V+: claims 5, 8, 24-26, and also claims 6 and 21 (in part) are drawn to methods for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof comprising administering to said subject an effective amount of an antagonist that inhibits activity of IL-18 Binding Protein (IL-18BP).

An exemplary election of Group V+ is a DR-18 comprising SEQ ID NO: 64, and methods for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof comprising the same. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named exemplary election was selected based on the first listed compound species presented in the claims (see claim 26).

The inventions listed in Groups I-V+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Group V+ formulas do not share a significant structural element responsible for DR-18 molecules requiring the selection of alternative amino acid sequences "wherein the DR-18 comprises an amino acid sequence selected from SEQ ID NOs: 64-97."

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

The special technical features of Group I, methods for ameliorating disease activity in autoimmune, allergic, or post-transplant disorders in a subject in need thereof, are not present in Groups II-V+; the special technical features of Group II, methods for treating an autoimmune disease caused by a harmful pro-inflammatory response to a self-antigen in a subject in need thereof, are not present in Groups I, or III-V+; the special technical features of Group III, methods for treating an inflammatory disease in a subject, are not present in Groups I, II, IV, or V+; the special technical features of Group IV, methods for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof comprising administering to said subject an effective amount of a therapeutic population of cells, are not present in Groups I-III, or V+, and the special technical features of Group V+, methods for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof comprising administering to said subject an effective amount of an antagonist that inhibits activity of IL-18 Binding Protein (IL-18BP), are not present in Groups I-IV.

Additionally, even if Groups I-V+ were considered to share the technical features of a method for treating an autoimmune disease; an agent that enhances the immune protective effects of Interleukin-18 (IL-18); a method for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof, comprising administering to said subject an effective amount of an antagonist that inhibits activity of IL-18 Binding Protein (IL-18BP), thereby increasing at least one of bioactivity and enhancement of immune protective effects of free IL-18 in said subject, thereby suppressing immunopathology resulting from the pathogenic CD4+ T cells and alleviating inflammatory symptoms in said subject, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2002/0068057 A1 to Feldmann et al. a method for treating an autoimmune disease (A method of treating autoimmune or inflammatory disease in a mammal comprising administering to said mammal a therapeutically effective amount of a combination of a CD4+ T cell inhibiting agent and a TNF antagonist, Claim 1).

Further, US 2019/0070262 A1 to Yale University teaches an agent that enhances the immune protective effects of Interleukin-18 (IL-18) (The potential advantage of such an agent is that it would serve to neutralize IL-18BP and enhance the activity of endogenous IL-18, Para. [0359]); and a method for treating a disease, comprising administering to said subject an effective amount of an antagonist that inhibits activity of IL-18 Binding Protein (IL-18BP), thereby increasing at least one of bioactivity and enhancement of immune protective effects of free IL-18 in said subject (a method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject a composition comprising the IL-18 binding protein (IL-18BP) inhibitor or IL-18BP antagonist, Para. [0033]; The potential advantage of such an agent is that it would serve to neutralize IL-18BP and enhance the activity of endogenous IL-18, Para. [0359]).

Further, US 2012/0263764 A1 to Watson teaches a method for treating an inflammatory disease caused by pathogenic CD4+ T cells in a subject in need thereof, comprising administering to said subject an agent, thereby suppressing immunopathology resulting from the pathogenic CD4+ T cells and alleviating inflammatory symptoms in said subject (methods of treatment of inflammatory neurodegenerative disease... including but not limited to multiple sclerosis and to regulating or modulating neuroinflammation, by contacting cells and/or administering a therapeutic composition, Para. [0094]; Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS)... inflammatory responses initiated by autoreactive CD4+ T-cells can mediate injury to myelin, Paras. [0006]-[0010]; The term "treating" refers to, and includes, reversing, alleviating, inhibiting the progress of, or preventing a disease, disorder or condition, or one or more symptoms thereof, Para. [0163]).

The inventions listed in Groups I-V+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

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Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1, 6, 21**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.