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(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE INFLAMMATORY DISEASES

(57) Abstract: The present invention relates to methods and pharmaceutical compositions for the treatment of autoimmune inflammatory disease. In particular, the present invention relates to a method of treating an autoimmune inflammatory disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of at least one OX1R agonist.

WO 2017/064302 A1

METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE INFLAMMATORY DISEASES

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of autoimmune inflammatory diseases.

BACKGROUND OF THE INVENTION:

Inflammation is a coordinated process designed by evolution to eliminate pathogens and enable healing. However, this is carefully orchestrated in the sense that when it is no longer necessary, it must be actively terminated to avoid tissue damage and/or auto-immunity. In this line, if the activities of the pro-inflammatory IFN-γ (or Th1) and IL-17 (or Th17) producing T helper cells are not efficiently modulated after host defense, these T cell subsets contribute to autoimmune inflammatory conditions such as multiple sclerosis (MS), inflammatory bowel disease (IBD) and chronic pancreatitis. For instance, inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that comprises Crohn's disease (CD) and ulcerative colitis (UC). For a long time, CD was considered to be Th1-driven and UC Th2-driven, but more recently Th17 cells may participate in their pathogeneses. In the same way, chronic pancreatitis which is a progressive inflammatory disease of the pancreas leading to inflammation and fibrosis associated to the exocrine and endocrine insufficiency, is characterized by a predominance of Th1 response. In particular, chronic pancreatitis is a progressive inflammatory disease which leads to the permanent deterioration of the structure and function of the pancreas characterized by inflammation, fibrosis and exocrine/endocrine insufficiency. Multiple sclerosis is a chronic demyelinating disease of the central nervous system. Despite its complex pathogenesis, evidence supports an autoimmune component of the disease driving chronic inflammatory processes in the spinal cord and brain. Although it was classically considered that the nervous and immune systems were independent from each other, it is now known that they interact through common mediators and receptors. In this sense, the list of neuropeptides that exert immunomodulatory properties is continuously growing. Orexin A and orexin B (also known as hypocretin 1 and hypocretin 2, respectively), are two neuropeptides derived from a common precursor polypeptide, which were initially identified as endogenous ligands for two orphan G protein-coupled receptors (OX1R and OX2R). Originally discovered in the hypothalamus, they are mainly known for their ability to regulate sleep and

arousal states, appetite and feeding, gastrointestinal mobility and energy homeostasis. The potential involvement of orexin in the immune system has been barely investigated.

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of autoimmune inflammatory diseases such as multiple sclerosis. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

In order to test a potential therapeutic value of orexin A in autoimmune inflammatory diseases such as inflammatory bowel diseases, chronic pancreatitis and multiple sclerosis, the inventors have interestingly found, that orexin A significantly ameliorated the clinical features in a model of colitis (i.e. the DSS model) in a model of chronic pancreatitis and in a model of multiple sclerosis (i.e. the EAE model). Accordingly, OX1R agonist would thus be suitable for the treatment of autoimmune inflammatory diseases.

Accordingly an object of the present invention relates to a method of treating an autoimmune inflammatory disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of at least one OX1R agonist.

As used herein, the term "subject" denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a subject according to the invention is a human.

As used herein, the expression "autoimmune inflammatory disease" is used herein in the broadest sense and includes all diseases and pathological conditions where the pathogenesis of which involves abnormalities of Th1 and Th17 cells, in particulate accumulation of Th1 and Th17 cells in organs. As used herein, the term "Th17 cells" has its general meaning in the art and refers to a subset of T helper cells producing interleukin 17 (IL-17). "A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage". Nat. Med. 13 (2): 139-145, 2007). The term "IL-17" has its general meaning in the art and refers to the interleukin-17A protein. Typically, Th17 cells are characterized by classical expression of Th cell markers at their cell surface such as CD4, and by the expression of IL17. Typically, as referenced herein, a Th17 cell is a IL-17+ cell. As used herein, the term "Th1 cell"

mean a type-1 helper T cell characterized by classical expression of CD4 and its ability to produce high levels of the proinflammatory cytokine IFNγ.

In particular, the above-mentioned autoimmune inflammatory diseases may be one or more selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid,

alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome

(OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital

rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy,

Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

In some embodiments, the method of the present invention is particularly suitable for the treatment of multiple sclerosis.

As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), preventing or delaying the spread of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. The term “treatment” encompasses the prophylactic treatment. As used herein, the term "prevent” refers to the reduction in the risk of acquiring or developing a given condition, or the reduction or inhibition of the recurrence or said condition in a subject who is not ill, but who has been or may be near a subject with the disease.

As used herein, the term “OX1R” has its general meaning in the art and refers to the 7-transmembrane spanning receptor OX1R for orexins. An exemplary amino acid sequence of OX1R is shown as SEQ ID NO:1.

SEQ ID NO:1: human orexin receptor-1 OX1R

MEPSATPGAQ MGVPPGSREP SPVPPDYEDE FLRYLWRDYL YPKQYEWVLI AAYVAVFVVA LVGNTLVCLA VWRNHHMRTV TNYFIVNLSL ADVLVTAICL PASLLVDITE SWLFGHALCK VIPYLQAVSV SVAVLTLSFI ALDRWYAICH PLLFKSTARR ARGSILGIWA VSLAIMVPQA AVMECSSVLP ELANRTRLFS VCDERWADDL YPKIYHSCFF IVTYLAPLGL MAMAYFQIFR KLWGRQIPGT TSALVRNWKR PSDQLGDLEQ GLSGEPQPRG RAFLAEVKQM RARRKTAKML MVVLLVFALC YLPISVLNVL KRVFGMFRQA SDREAVYACF TFSHWLVYAN SAANPIIYNF LSGKFREQFK AAFSCCLPGL GPCGSLKAPS PRSSASHKSL SLQSRCSISK ISEHVVLTSV TTVLP

Accordingly, as used herein, the term "OX1R agonist" refers to any compound natural or not that is able to bind to OX1R and promotes OX1R activity. In particular, an easy method for determining whether a compound is an OX1R agonist consists in determining whether the candidate is able to induce a transient calcium release in a cell expressing OX1R.

In some embodiments, the OX1R agonist is a small organic molecule. The term "small organic molecule" refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e. g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, more in particular up to 2000 Da, and most in particular up to about 1000 Da.

In some embodiment, the OX1R agonist is an anti-OX1R antibody.

As used herein, "antibody" includes both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or non-human antibody. A non-human antibody may be humanized by recombinant methods to reduce its immunogenicity in man.

In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the portion of the antibody comprises a light chain of the antibody. In some embodiments, the

portion of the antibody comprises a heavy chain of the antibody. In some embodiments, the portion of the antibody comprises a Fab portion of the antibody. In some embodiments, the portion of the antibody comprises a F(ab')2 portion of the antibody. In some embodiments, the portion of the antibody comprises a Fc portion of the antibody. In some embodiments, the portion of the antibody comprises a Fv portion of the antibody. In some embodiments, the portion of the antibody comprises a variable domain of the antibody. In some embodiments, the portion of the antibody comprises one or more CDR domains of the antibody.

Antibodies are prepared according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of OX1R. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes. Briefly, the recombinant OX1R may be provided by expression with recombinant cell lines. In particular, OX1R may be provided in the form of human cells expressing OX1R at their surface. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology,* 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope. In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDRS). The CDRs, and in particular the CDRS regions, and more particularly the heavy chain CDRS, are largely responsible for antibody specificity. It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization

include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567,5,225,539,5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may be used in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid reside at the framework positions at which the amino acid is predicted to have a side chain atom within 3A of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In some embodiments of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgGl, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., /. *Mol. Biol.* 294:151, 1999, the contents of which are incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been

genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab') 2 Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgGl, IgG2, IgG3 and IgG4.

In some embodiments, the antibody according to the invention is a single domain antibody. The term “single domain antibody” (sdAb) or "VHH" refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are

naturally devoid of light chains. Such VHH are also called “nanobody®”. According to the invention, sdAb can particularly be llama sdAb.

In some embodiments, the OX1R agonist is a polypeptide. In some embodiments the polypeptide is a functional equivalent of Orexin-A or Orexin-B.

As used herein the term “Orexin-A” has its general meaning in the art and refers to the amino acid sequence as shown by SEQ ID NO:2.

Orexin-A: peplpdccrqk tcscrlyell hgagnhaagi ltlx (SEQ ID NO:2)

As used herein the term “orexin-B” has its general meaning in the art and refers to the amino acid sequence as shown by SEQ ID NO:3.

Orexin-B: fsgppglqgr lqrllqasgn haagiltm (SEQ ID NO:3) or glqgr lqrllqasgn haagiltm (SEQ ID NO:4).

As used herein, a “functional equivalent of orexin” is a polypeptide which is capable of binding to OX1R, thereby promoting an OX1R activity according to the invention. The term "functional equivalent" includes fragments, mutants, and muteins of Orexin-A and Orexin-B. The term "functionally equivalent" thus includes any equivalent of orexins (i.e. Orexin-A or Orexin-B) obtained by altering the amino acid sequence, for example by one or more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to OX1R and promote an OX1R activity according to the invention. Amino acid substitutions may be made, for example, by point mutation of the DNA encoding the amino acid sequence.

In some embodiments, the functional equivalent is at least 70% of identity to the corresponding protein. According to the invention a first amino acid sequence having at least 70% of identity with a second amino acid sequence means that the first sequence has 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; or 99% of identity with the second amino acid sequence and conserving biological properties of said second amino acid sequence. Amino acid sequence identity is preferably

determined using a suitable sequence alignment algorithm and default parameters, such as BLAST P (Karlin and Altschul, 1990).

The term "a functionally equivalent fragment" as used herein also may mean any fragment or assembly of fragments of Orexin that binds to OX1R and promote the OX1R activity according to the invention. Accordingly the present invention provides a polypeptide which comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of Orexin-A or Orexin-B, which portion binds to OX1R and promotes the OX1R activity according to the invention.

The polypeptides of the invention may be produced by any suitable means, as will be apparent to those of skill in the art. In order to produce sufficient amounts of polypeptides or functional equivalents thereof for use in accordance with the present invention, expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the polypeptide of the invention. In particular, the polypeptide is produced by recombinant means, by expression from an encoding nucleic acid molecule. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. When expressed in recombinant form, the polypeptide is in particular generated by expression from an encoding nucleic acid in a host cell. Any host cell may be used, depending upon the individual requirements of a particular system. Suitable host cells include bacteria mammalian cells, plant cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells. HeLa cells, baby hamster kidney cells and many others. Bacteria are also preferred hosts for the production of recombinant protein, due to the ease with which bacteria may be manipulated and grown. A common, preferred bacterial host is *E coli.*

In some embodiments, the polypeptide of the invention is an immunoadhesin.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin" which is able to bind to OX1R) with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity to OX1R (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid

sequence comprising at least the binding site for OX1R. In some embodiments, the adhesin comprises the polypeptides characterized by SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

The immunoglobulin sequence typically, but not necessarily, is an immunoglobulin constant domain (Fc region). Immunoadhesins can possess many of the valuable chemical and biological properties of human antibodies. Since immunoadhesins can be constructed from a human protein sequence with a desired specificity linked to an appropriate human immunoglobulin hinge and constant domain (Fc) sequence, the binding specificity of interest can be achieved using entirely human components. Such immunoadhesins are minimally immunogenic to the patient, and are safe for chronic or repeated use.

In some embodiments, the Fc region is a native sequence Fc region. In some embodiments, the Fc region is a variant Fc region. In still another embodiment, the Fc region is a functional Fc region. As used herein, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The adhesion portion and the immunoglobulin sequence portion of the immunoadhesin may be linked by a minimal linker. The immunoglobulin sequence typically, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD or IgM, but typically IgG1 or IgG3.

The polypeptides of the invention, fragments thereof and fusion proteins (e.g. immunoadhesin) according to the invention can exhibit post-translational modifications, including, but not limited to glycosylations, (e.g., N-linked or O-linked glycosylations), myristylations, palmitylations, acetylations and phosphorylations (e.g., serine/threonine or tyrosine).

In some embodiments, it is contemplated that polypeptides used in the therapeutic methods of the present invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. In example adding dipeptides can improve the penetration of a circulating agent in the eye through the blood retinal barrier by using endogenous transporters.

A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect, water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain.

Polyethylene glycol (PEG) has been widely used as a drug carrier, given its high degree of biocompatibility and ease of modification. Attachment to various drugs, proteins, and liposomes has been shown to improve residence time and decrease toxicity. PEG can be coupled to active agents through the hydroxyl groups at the ends of the chain and via other chemical methods; however, PEG itself is limited to at most two active agents per molecule. In a different approach, copolymers of PEG and amino acids were explored as novel biomaterials which would retain the biocompatibility properties of PEG, but which would have the added advantage of numerous attachment points per molecule (providing greater drug loading), and which could be synthetically designed to suit a variety of applications.

Those of skill in the art are aware of PEGylation techniques for the effective modification of drugs. For example, drug delivery polymers that consist of alternating polymers of PEG and tri-functional monomers such as lysine have been used by VectraMed (Plainsboro, N.J.). The PEG chains (typically 2000 daltons or less) are linked to the a- and e-amino groups of lysine through stable urethane linkages. Such copolymers retain the desirable properties of PEG, while providing reactive pendent groups (the carboxylic acid groups of lysine) at strictly controlled and predetermined intervals along the polymer chain. The reactive pendent groups can be used for derivatization, cross-linking, or conjugation with other molecules. These

polymers are useful in producing stable, long-circulating pro-drugs by varying the molecular weight of the polymer, the molecular weight of the PEG segments, and the cleavable linkage between the drug and the polymer. The molecular weight of the PEG segments affects the spacing of the drug/linking group complex and the amount of drug per molecular weight of conjugate (smaller PEG segments provides greater drug loading). In general, increasing the overall molecular weight of the block co-polymer conjugate will increase the circulatory half-life of the conjugate. Nevertheless, the conjugate must either be readily degradable or have a molecular weight below the threshold-limiting glomular filtration (e.g., less than 60 kDa).

In addition, to the polymer backbone being important in maintaining circulatory half-life, and biodistribution, linkers may be used to maintain the therapeutic agent in a pro-drug form until released from the backbone polymer by a specific trigger, typically enzyme activity in the targeted tissue. For example, this type of tissue activated drug delivery is particularly useful where delivery to a specific site of biodistribution is required and the therapeutic agent is released at or near the site of pathology. Linking group libraries for use in activated drug delivery are known to those of skill in the art and may be based on enzyme kinetics, prevalence of active enzyme, and cleavage specificity of the selected disease-specific enzymes. Such linkers may be used in modifying the protein or fragment of the protein described herein for therapeutic delivery.

In some embodiments, the OX1R agonist is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods.

The terms "administer" or "administration" refer to the act of injecting or otherwise physically delivering a substance as it exists outside the body (e.g., a OX1R agonist of the present invention) into the subject, such as by mucosal, intradermal, intravenous, subcutaneous,

intramuscular delivery and/or any other method of physical delivery described herein or known in the art. When a disease, or a symptom thereof, is being treated, administration of the substance typically occurs after the onset of the disease or symptoms thereof. When a disease or symptoms thereof, are being prevented, administration of the substance typically occurs before the onset of the disease or symptoms thereof.

In some embodiments, the OX1R agonist of the invention is administered to the subject with a therapeutically effective amount.

By a "therapeutically effective amount" is meant a sufficient amount of OX1R to treat the autoimmune inflammatory disease at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. In particular, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, in particular from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

The OX1R agonist of the invention is typically combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to be administered in the form of a pharmaceutical composition. "Pharmaceutically"

or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The antibody can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium,

potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active antibody in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Orexin A ameliorates the DAI (weight and colitis score) of DSS-induced colitis mice. Mice were orally treated with 5% DSS for 7 days in the presence or in the absence of daily intraperitoneal injection of OxA (0.22 µmoles/kg). DAI score including weight loss, severity of diarrhea, the presence of blood in the stool, inflammation and edema was estimated using a scale ranging from 0 (no symptoms) to 4 (severe colitis). DSS, dextran sulfate sodium; WT, wild type mice.

Figure 2. Preventive and curative Orexin A treatments dramatically alleviate EAE symptoms. EAE was induced with 100 µg of MOG_{35-55} (Myelin Oligodendrocyte Glycoprotein 35-55) as previously described (Proc Natl Acad Sci U S A. 106(6):2012-7, 2009) to 9 week old female C57BL/6 wild-type (WT) mice (n=10/group). Panels show clinical signs of EAE scored daily in a blinded fashion for over 30 days on a scale of 0-5 as follows: 0, no detectable clinical signs, 1, waddling gait with limp tail, 2, ataxia with full paralysis of one limb, 3, full paralysis of two limbs, 4, full paralysis of two limbs with important weight loss (>20% of initial weight) and 5, moribund or dead. Then, mice were given:

Panel A, intraperitoneally (IP) either PBS (group PBS IP), 100 µg of orexin A per mouse for 5 days on day 3 (= before the onset, group $BOxA_{100}$ IP) or at a moderate EAE score (= 1,5-2 for group OxA_{100} IP).

Panel B, intraperitoneally either PBS (group PBS), 100 µg (group OxA_{100} IP) or 300 µg (group OxA_{300} IP) of orexin A per mouse for 5 days at a moderate EAE score (= 1,5-2).

Panel C, either PBS (group PBS), 300 µg of orexin A per mouse intraperitoneally (group OxA_{300} IP) or retro-orbitally (RO) (group OxA_{300} RO) for 5 days at a moderate EAE score (= 1,5-2).

The EAE score is shown as mean +/- SEM. *$P<0.05$ (nonparametric t-test, compared to PBS group).

Figure 3. Orexin A significantly decreases histopathological score of EAE mice. 30 days post-immunization, mice (from the PBS, OxA_{300} RO, and OxA_{300} IP groups of Figure 2,

panel C) were sacrificed and spinal cords were harvested, fixed overnight in 4% paraformaldehyde and stored in 70% ethanol. Spinal cords were then embedded in paraffin and cut in 0.7 µm section. After haematoxylin/eosin (to distinguish cell infiltration) and luxol fast blue (to label myelin) staining, photography was performed at x1,25 and x10 magnifications for the spinal cord sections of PBS, OxA_{300} RO and OxA_{300} IP mice. The graph shows the mean scores of each group (n=5/group). Histopathology grading was as follows: 0-normal appearance, 1- some infiltrated cells and low demyelination, 2- 2 or 3 infiltrated areas and low demyelination, 3- numerous infiltrated areas and strong demyelination, 4- important cell infiltration throughout the tissue with strong demyelination. *P<0.05 (nonparametric t-test, compared to PBS group).

Figure 4. Orexin A efficiently suppresses Th1-specific (IFNγ) and Th17-specific (IL-17) cytokine gene expression in the brain of EAE mice. It was determined 30 days after EAE induction of PBS, OxA_{300} RO, and OxA_{300} IP mice (from Figure 2, panel C) by real time RT-qPCR as previously described (Proc Natl Acad Sci U S A. 106(6):2012-7, 2009). *P<0.05 (nonparametric t-test, compared to PBS group).

Figure 5. Orexin A-treated mice exhibit increased regulatory T cell (Treg) proportion in comparison with PBS-treated mice in draining lymph nodes during EAE. On day 30 post-immunization, draining lymph nodes were harvested from the naïve, PBS, OxA_{300} RO, and OxA_{300} IP groups (Figure 2, panel C). Assessment by flow cytometry was performed by using the mouse regulatory T cell staining kit: lymph node Tregs were defined as $CD4^+CD25^+Foxp3^+$cells and proliferative Tregs as $CD4^+CD25^+Foxp3^+Ki67^+$cells.

Histograms represent the mean of the percentage (left) and the total number (right) of Tregs and proliferative Tregs ($Ki67^+$Tregs) for each group. Bars represent the mean +/- SEM of 5 individual mice. *P<0.05 (non parametric t-test, compared to PBS group).

Figure 6. Scoring of OX1R expression in normal pancreas and pancreatitis in human. OX1R expression was determined by immunohistochemistry using anti-OX1R antibody. Scoring of slices was determined as the intensity of OX1R expression (0 to 3) x the percentage of labelled pancreatitis surface (0 to 100%).

Figure 7. Pancreatic lymphocyte infiltration determined by immunostaining of CD45+ cells. Values were expressed as percentage of stained surface. **, p<0.01

Figure 8. Amylase activity in blood samples of control mice, cerulein-induced mice and cerulein-induced mice treated with OxA. *, $p<0.05$; ***, $p<0.001$ and NS = non-significant.

EXAMPLE 1:

Orexin A ameliorates the DAI (weight and colitis score) of DSS-induced colitis mice. As shown in Figure 1 when DSS-induced colitis mice were treated with OxA, the DAI score was ameliorated (about of 1.5). We have also found that orexin A administration to mice undergoing chronic experimental autoimmune encephalomyelitis (EAE) (a widely used mouse model for progressive MS) significantly ameliorated the clinical features of the disease at a dose-dependent fashion (Figure 2). Interestingly, this result was accompanied with drastic reduction of the histopathological EAE score (Figure 3) and of the Th1/Th17 pro-inflammatory responses (Figure 4) in the CNS tissues, but with an increase of regulatory T cell (Treg, which play a critical role during inflammation) proportion (Figure 5) in orexin A treated-mice versus PBS controls. Therefore, orexin A presents potent intrinsic anti-inflammatory properties, capable of modulating the Th/Treg homeostasis during an auto-immune response as aggressive as in a chronic EAE model.

EXAMPLE 2:

Chronic pancreatitis is a progressive inflammatory disease which leads to the permanent deterioration of the structure and function of the pancreas characterized by inflammation, fibrosis and exocrine/endocrine insufficiency. Orexins (orexin-A and orexin-B) are hypothalamic peptides involved in the sleep/wake control which interact with two GPCR sub-types, OX1R and OX2R. We have recently observed that OX1R is highly expressed in the whole pancreas in human pancreatitis (Figure 6), whereas it is restricted to islets in the normal pancreas. Moreover, we have demonstrated the anti-inflammatory role of orexin in colitis.

We have investigated the effect of orexin A (OxA) on chronic pancreatitis mice model induced by supraphysiologic doses of cerulein (3 intraperitoneal injections/week at 100µg/kg). After 3 weeks of cerulein, histological analysis of the pancreas revealed fibrosis, chronic inflammation and acino-ductal metaplasia. In mice treated by OxA (2 intraperitoneal injections/week at 1.40 µmol/Kg) the lesions induced by cerulein improved. OxA-treated mice had lower number of acino-ductal metaplasia. OxA treatment lowered pancreatic fibrosis on Picrosirius staining (12% of the pancreatic surface, as compared to 40% in OxA-untreated mice

evaluated by quantitative imaging analysis). OxA treatment reduced pancreatic lymphocyte infiltration evaluated by immunohistochemistry with anti-CD45 antibody (6 % of the pancreatic surface, as compared to 18% in OxA-untreated mice evaluated by quantitative imaging analysis) (Figure 7). Finally the amylase activity is significantly reduced in OxA-treated mice (Figure 8).

These results demonstrate the protective role of orexin in the development of chronic pancreatitis induced by cerulein in a mice model. In conclusion, the orexins/OX1R system may represents an innovative and effective target in the treatment of pancreatitis.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A method of treating an autoimmune inflammatory disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of at least one OX1R agonist.

2. The method of claim 1 wherein the autoimmune inflammatory disease is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous

proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis

(Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous

angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies,

oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

3. The method of claim 1 wherein the autoimmune inflammatory disease is multiple sclerosis.

4. The method of claim 1 wherein the autoimmune inflammatory disease is chronic pancreatitis.

5. The method of claim 1 the OX1R agonist is selected from the group consisting of small organic molecules, antibodies, aptamers and polypeptides.

6. The method of claim 1 wherein the OX1R agonist is an antibody.

7. The method of claim 1 wherein the OX1R agonist is a polypeptide having at least 70% of identity with SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

8. The method of claim 1 wherein the OX1R agonist is a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

9. The method of claim 1 wherein the OX1R agonist is an immunoadhesin comprising a polypeptide characterized by SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 fused to a Fc domain.

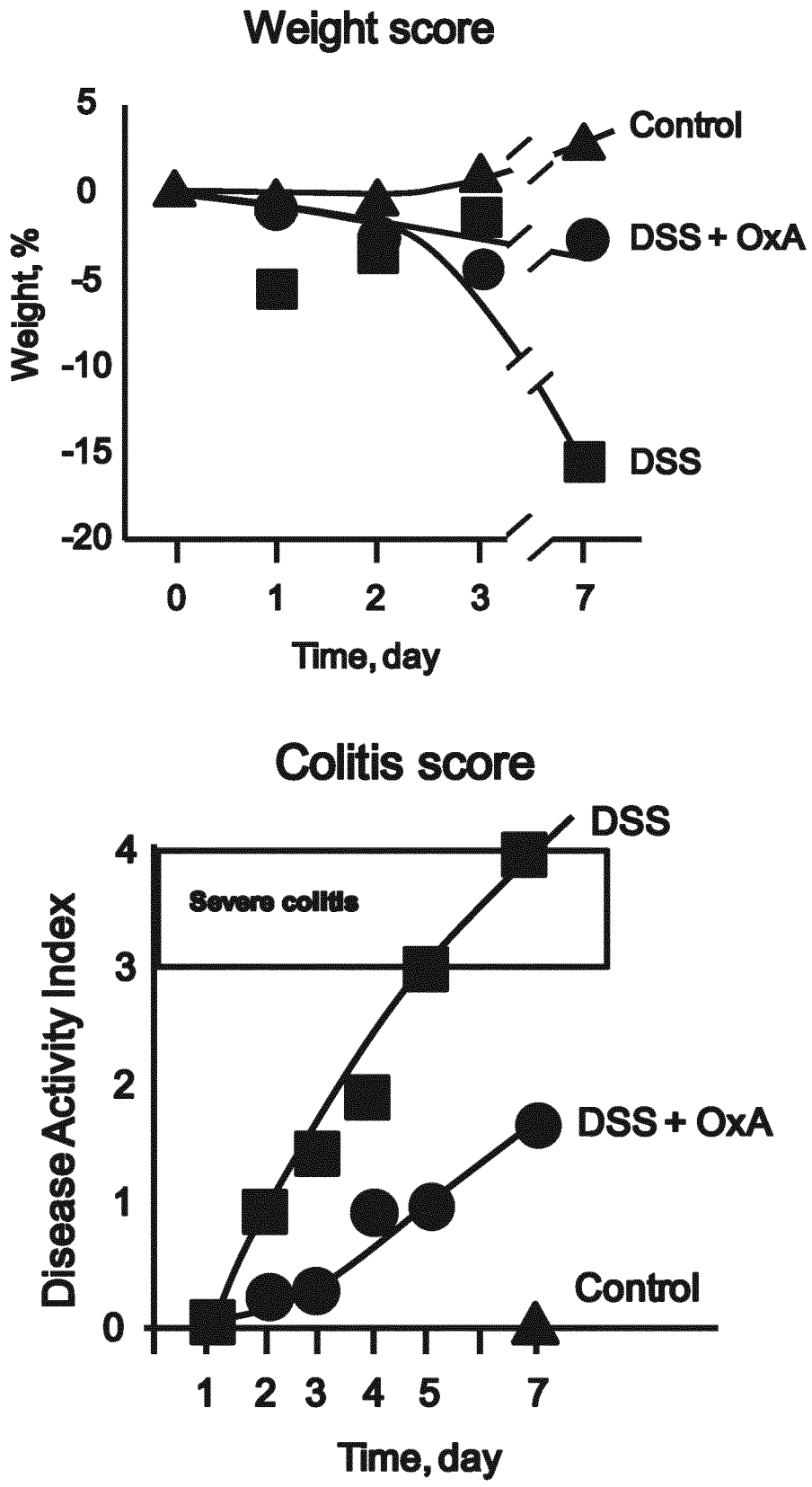
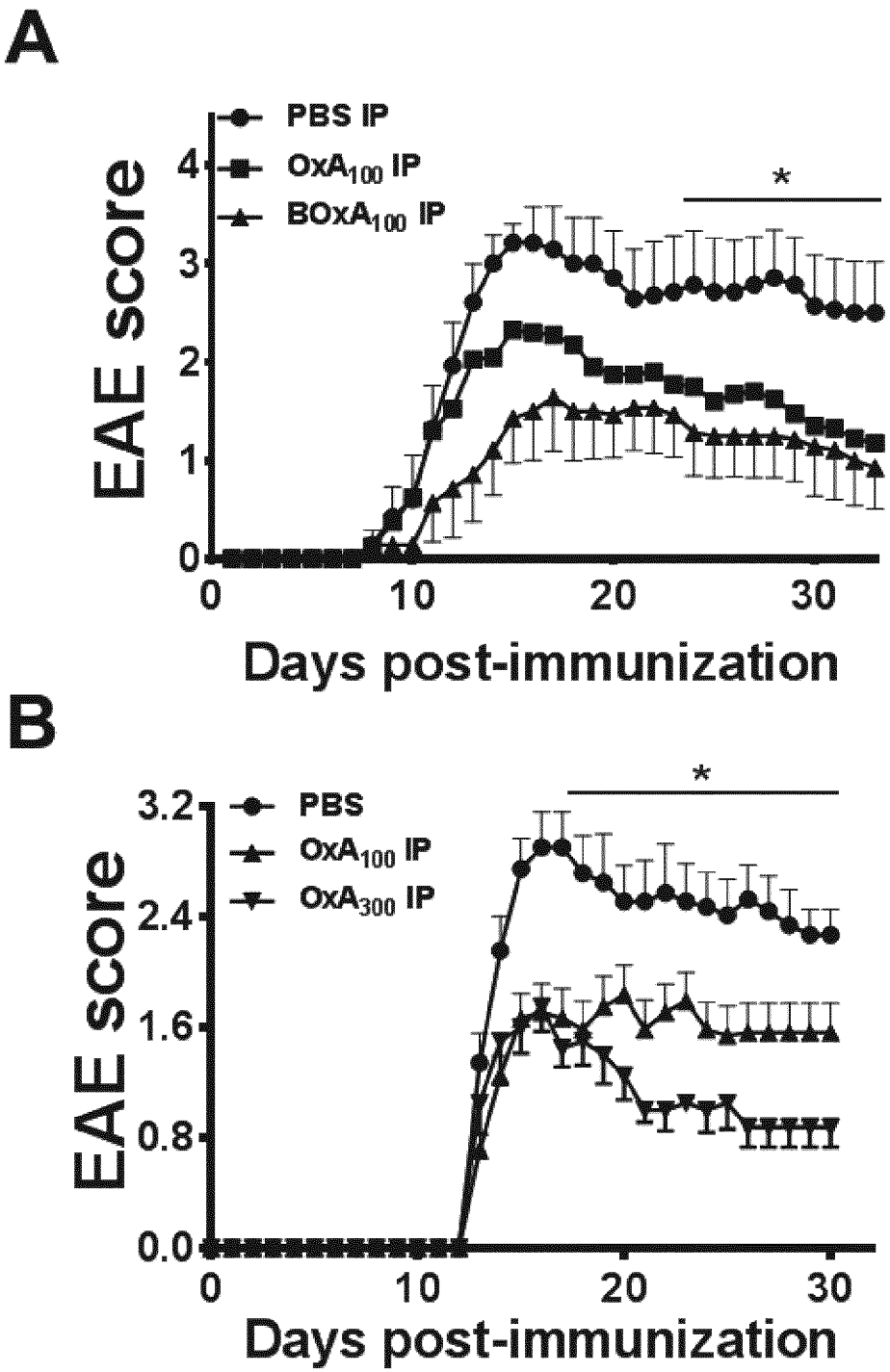

Weight score
5
0
-5
-10
-15
-20
Weight, %
Control
DSS + OxA
DSS
0
1
2
3
7
Time, day
Colitis score
DSS
4
Severe colitis
3
2
1
0
Disease Activity Index
DSS + OxA
Control
1
2
3
4
5
7
Time, day

Figure 1



Figures 2A and 2B

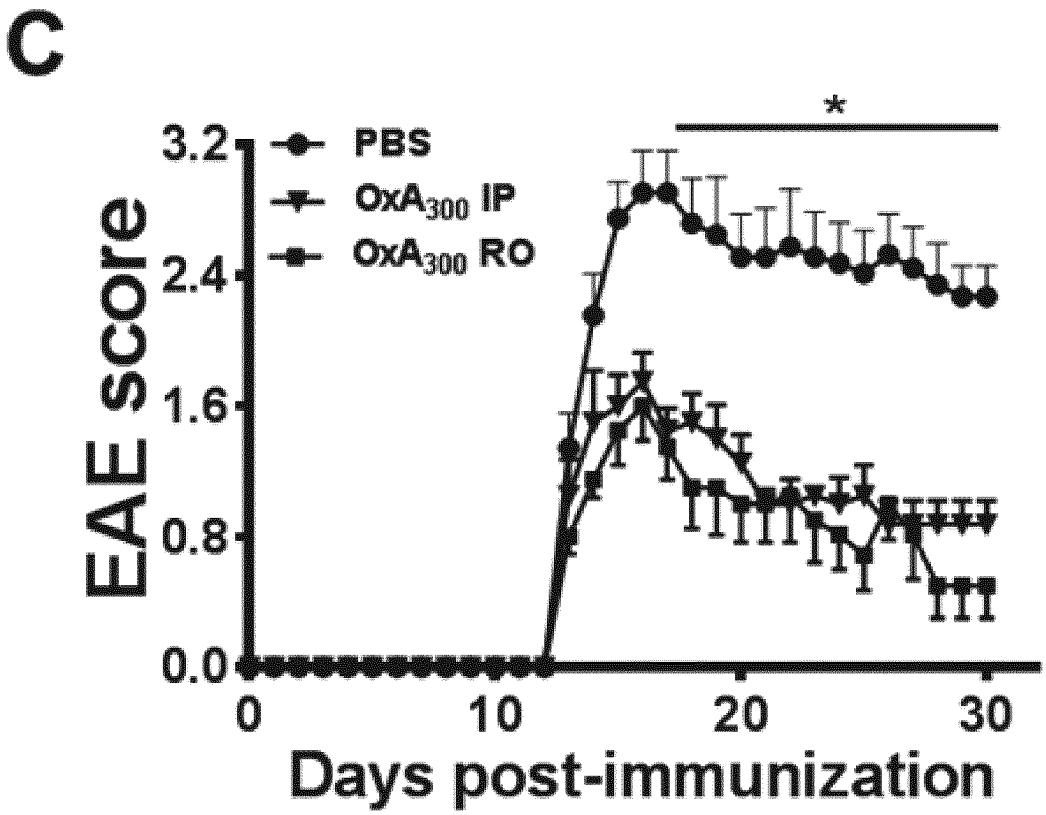

C
*
PBS
OxA300 IP
OxA300 RO
EAE score
3.2
2.4
1.6
0.8
0.0
0
10
20
30
Days post-immunization

Figure 2C

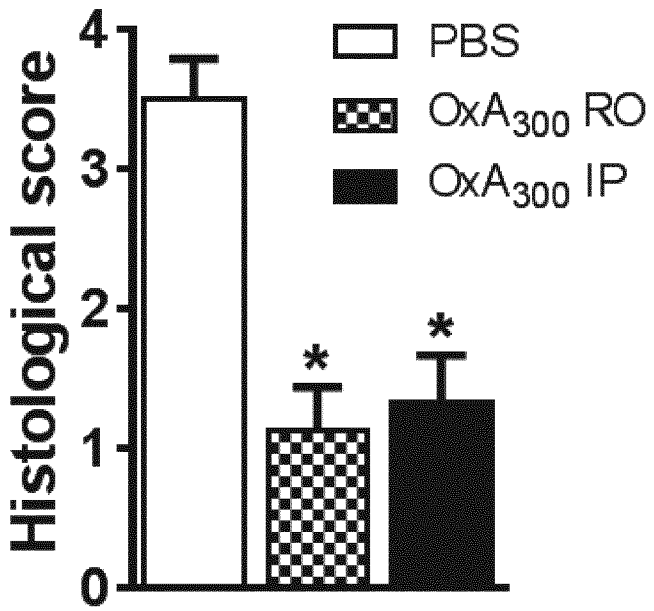


Figure 3

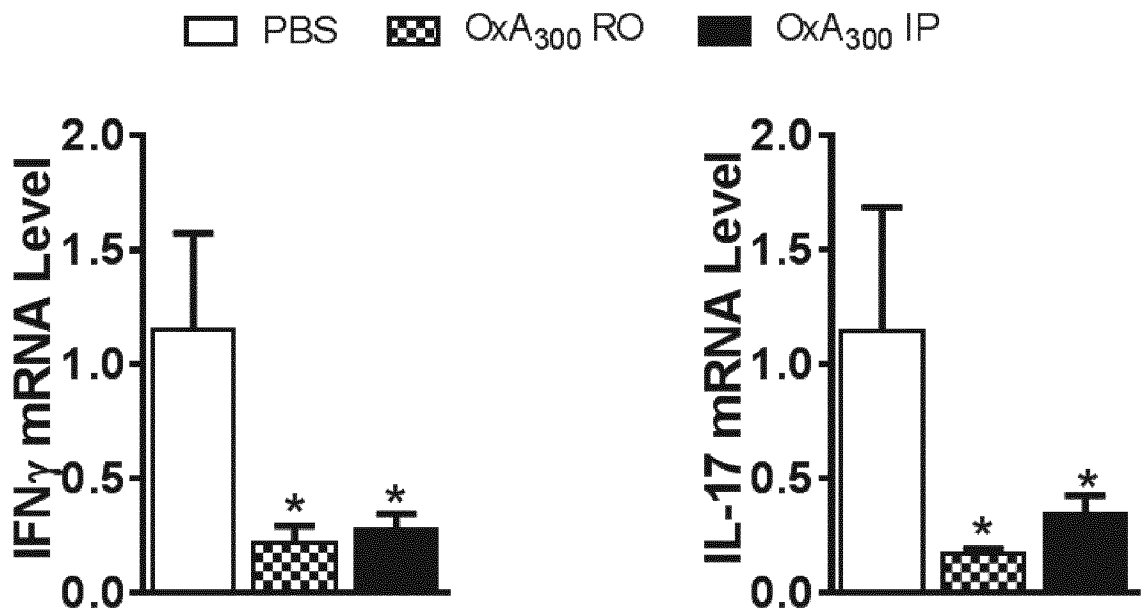


Figure 4

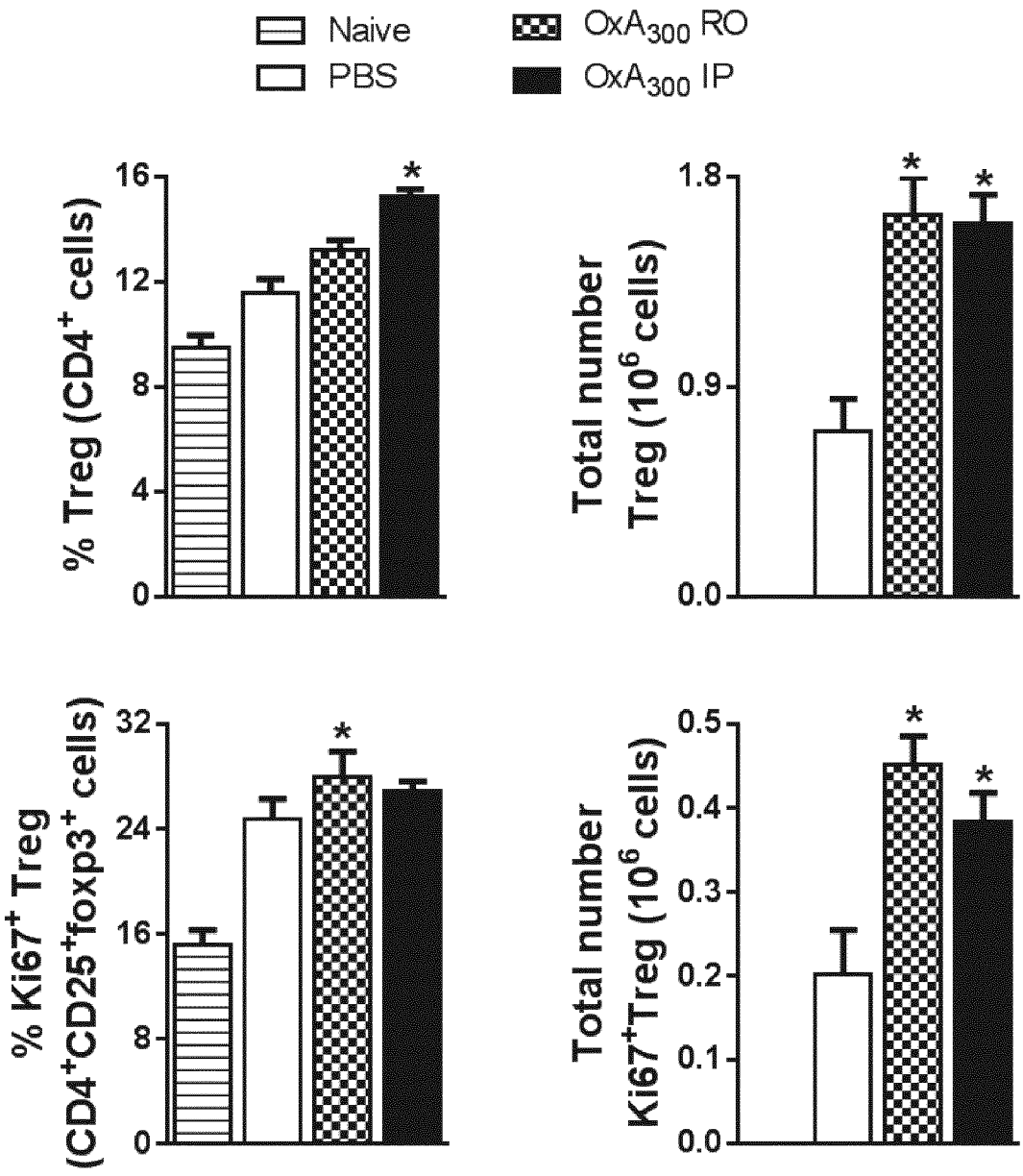

Naive
PBS
OxA300 RO
OxA300 IP
% Treg (CD4+ cells)
16
12
8
4
0
*
Total number Treg (10^6 cells)
1.8
0.9
0.0
% Ki67+ Treg (CD4+CD25+foxp3+ cells)
32
24
16
8
0
Total number Ki67+Treg (10^6 cells)
0.5
0.4
0.3
0.2
0.1
0.0

Figure 5

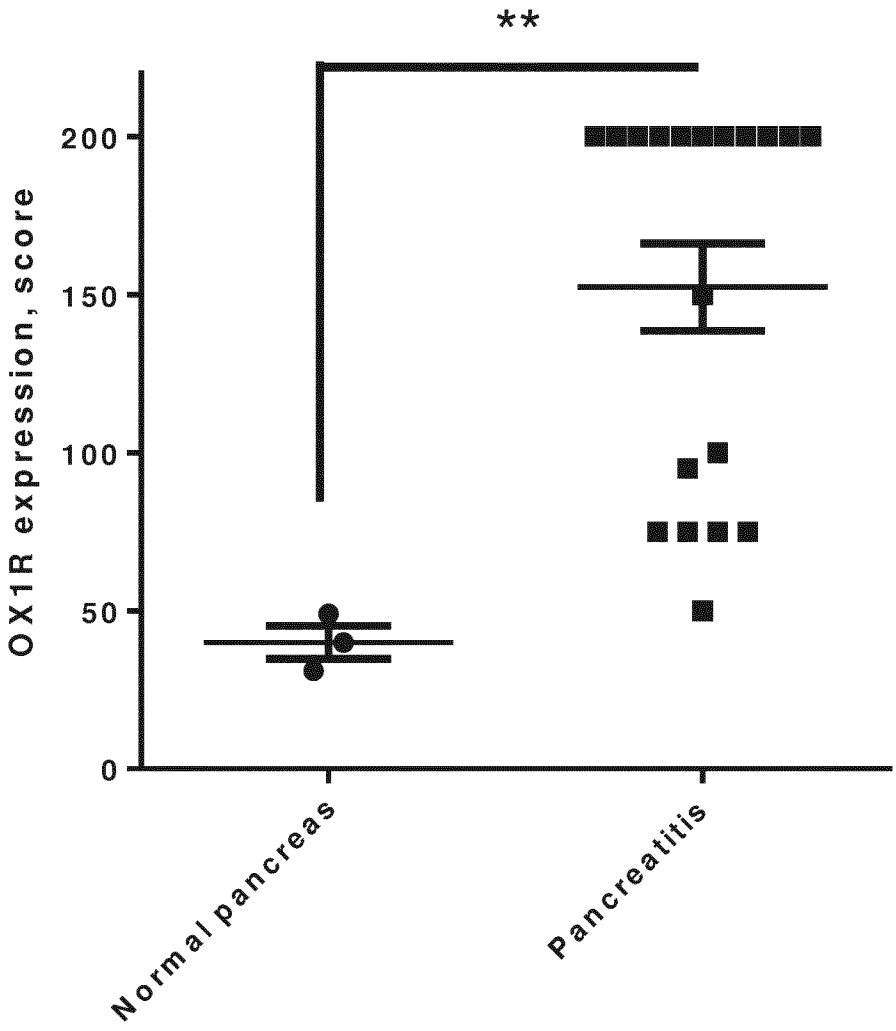

**
200
150
100
50
0
OX1R expression, score
Normal pancreas
Pancreatitis

Figure 6

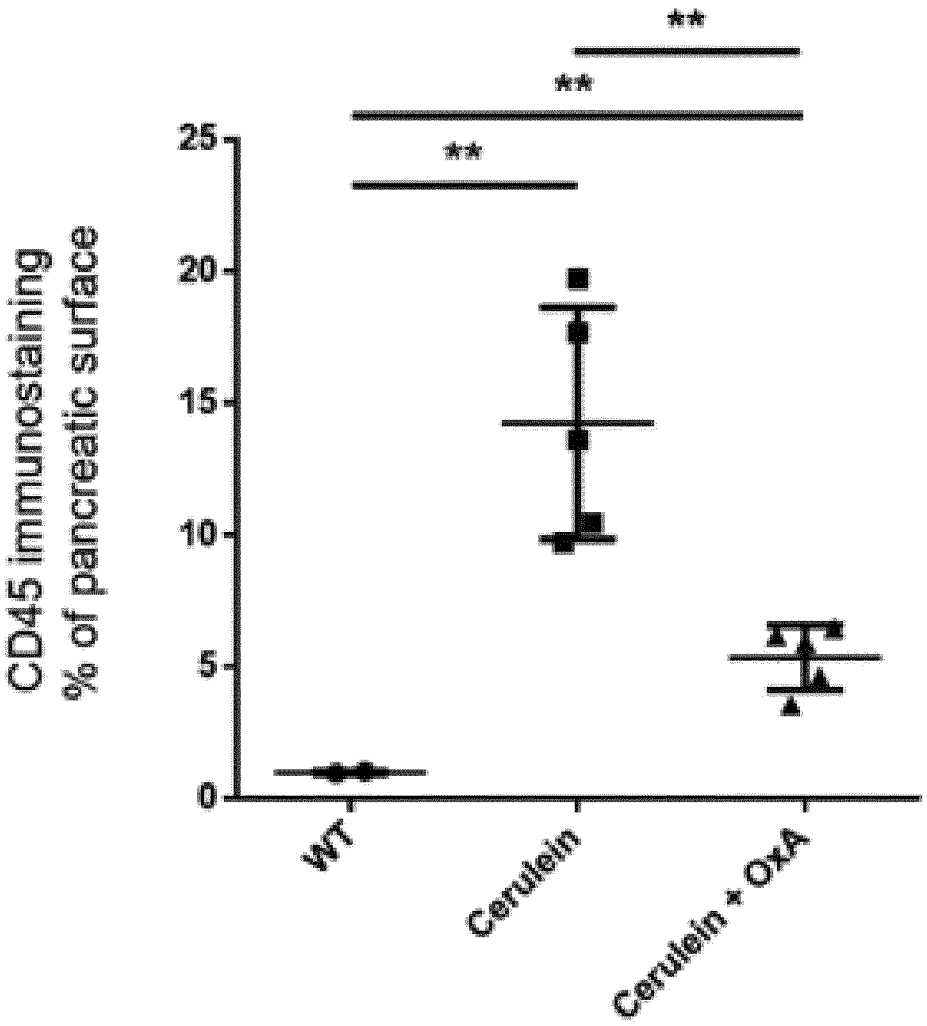

**
**
**
25
20
15
10
5
0
CD45 immunostaining
% of pancreatic surface
WT
Cerulein
Cerulein + OxA

Figure 7

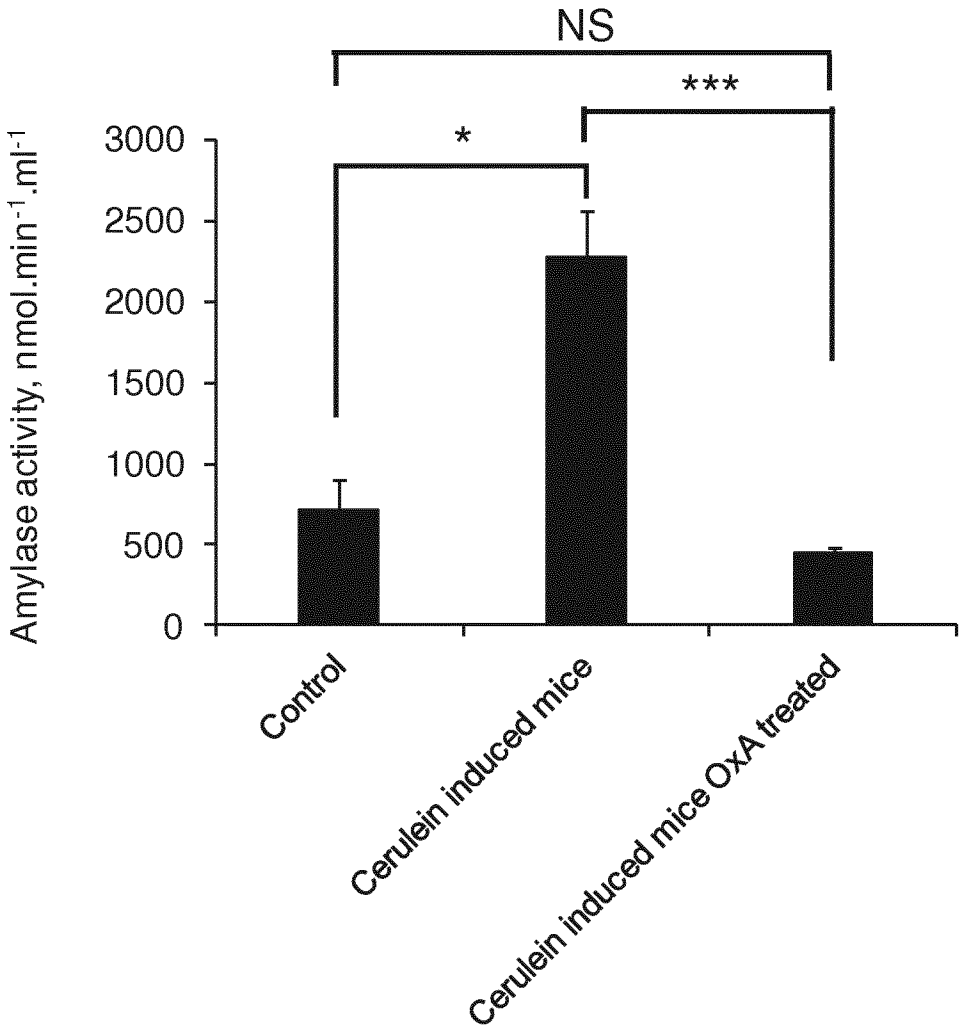

NS
***
*
3000
2500
2000
1500
1000
500
0
Amylase activity, nmol.min-1.ml-1
Control
Cerulein induced mice
Cerulein induced mice OxA treated

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/074806

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K38/22
ADD.

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)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIH-CHU CHIOU ET AL: "Orexins/Hypocretins: Pain Regulation and Cellular Actions", CURRENT PHARMACEUTICAL DESIGN, vol. 16, no. 28, 1 September 2010 (2010-09-01), pages 3089-3100, XP055150506, NL ISSN: 1381-6128, DOI: 10.2174/138161210793292483 the whole document -----	6-9
Y	WO 2008/128981 A1 (PROBIODRUG AG [DE]; BUCHHOLZ MIRKO [DE]; HEISER ULRICH [DE]; HAMANN AN) 30 October 2008 (2008-10-30) page 30 -----	6-9
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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

"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	Date of mailing of the international search report
7 February 2017	01/03/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Meyer, Wolfram

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/074806

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2015/197572 A1 (INSERM INST NAT DE LA SANTÉ ET DE LA RECH MÉDICALE [FR]; UNIVERSITÉ PA) 30 December 2015 (2015-12-30) the whole document -----	6-9
Y	DAVID ALEXANDRE ET AL: "The orexin type 1 receptor is overexpressed in advanced prostate cancer with a neuroendocrine differentiation, and mediates apoptosis", EUROPEAN JOURNAL OF CANCER, vol. 50, no. 12, 1 August 2014 (2014-08-01), pages 2126-2133, XP055196667, ISSN: 0959-8049, DOI: 10.1016/j.ejca.2014.05.008 the whole document -----	6-9

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/074806

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.: 1-5(completely); 6-9(partially)
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:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
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:

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 an 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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-5(completely); 6-9(partially)

1 Claims 1-5 do not meet enablement and/or support requirements (Article 5 PCT) considering the general scope of the claims. The claims encompass a genus of compounds ("agonist") defined by their function (acting as an agonist against OX1R) wherein the relationship between the structural features of the members of this genus and said function have not been defined. As no such relationship in the application as-filed, or which would have been recognized based upon information readily available to one skilled in the art, the skilled artisan would not know how and which compounds to use lacking structural definition. The fact that one could have assayed a compound of interest using the claimed assays does not overcome this defect since one would have no knowledge beforehand as to whether or not any given compound (other than those that might be particularly disclosed in the application) would fall within the scope of what is claimed. It would require undue experimentation (be an undue burden) to randomly screen undefined compounds and use them as "agonist".

2 The examiner is unable to search the all putative agonist in the method of treatment without undue burden. In the interests of clarity and for the purposes of an incomplete search, the applicant is requested to identify the subject-matter to be searched in more detail, for example, with reference to fully defined antagonist disclosed in the application and claims. The applicants comments will be considered when selecting the subject-matter of the search, and for defining the subject-matter of different inventions, should there be no common linking inventive concept found, in the sense of Rule 13 PCT.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/074806

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008128981 A1	30-10-2008	DK 2142513 T3	10-06-2014
		EP 2142513 A1	13-01-2010
		ES 2468551 T3	16-06-2014
		JP 5675340 B2	25-02-2015
		JP 2010524893 A	22-07-2010
		US 2008292582 A1	27-11-2008
		WO 2008128981 A1	30-10-2008
WO 2015197572 A1	30-12-2015	NONE	