Title: CD300LG POLYPEPTIDES AND THEIR USE IN TREATING AUTOIMMUNE DISEASES

Abstract: The present invention relates to novel CD300LG polypeptides, more specifically soluble forms thereof and provides use thereof and methods for preventing and/or treating diseases and/or disorders such as inflammatory diseases, including various inflammatory autoimmune disorders, allergic hypersensitivity, allograft rejection and/or syndromes associated with increased CD300LG-FL polypeptide function on dendritic cells.
CD300LG POLYPEPTIDES AND THEIR USE IN TREATING AUTOIMMUNE DISEASES

FIELD OF THE INVENTION

The present invention relates to novel CD300LG polypeptides, more specifically soluble forms thereof and provides use thereof and methods for preventing and/or treating diseases and/or disorders such as inflammatory diseases, including various inflammatory autoimmune disorders, allergic hypersensitivity, allograft rejection and/or syndromes associated with increased CD300LG-FL polypeptide function on dendritic cells.

BACKGROUND OF THE INVENTION

Dendritic cells are both able to initiate and suppress antigen-specific immune responses, depending on a variety of exogenous stimuli and the nature of the antigen, however, regulation of this balance is still poorly understood. Several cytokines are known to induce or sustain tolerogenic DCs, which are important to suppress overwhelming and unwanted activation of the immune system (e.g. to prevent autoimmunity) and to maintain the normal homeostasis of the entire organism. The best studied immunosuppressive, tolerogenic cytokines so far are IL-10 and TGF-β (Müller et al., 2002, J. Invest Dermatol. 119, 836-841.; Alard et al., 2004, Eur. J. Immunol. 34, 1021-1030), but also IL-21 has recently been shown to act as a suppressive, tolerogenic cytokine for dendritic cells (DCs) that inhibits DC maturation and function thereby inducing tolerogenic DCs. IL-21 induced tolerogenic cells are unable to induce in vitro and in vivo T-cell activation and T-cell dependent immune responses as contact-hypersensitivity but induce antigen-specific tolerance (Brandt et al., 2003, J. Invest Dermatol. 121, 1379-1382). Moreover, TNF-α is considered to induce suppressive, “semi-mature” DCs (Menges et al., 2002, J. Exp. Med. 195, 15-21). In this respect, regulatory T-cells have recently become the focus of intensive research, and most in vivo models of T_{reg} cell suppression have provided strong evidences for bystander suppression and dependence on IL-10 or TGF-β. More recently, it was demonstrated that the suppressive action of T_{reg} is not directed to other (effector) T-cells but rather to DCs (Tang et al., 2006). A possibility therefore is that T_{reg} secrete tolerogenic cytokines including IL-21, IL-10, TNF-α and TGF-β, to modify the DC phenotype and/or activation state. As it has been shown for all four cytokines in different in vivo models, DCs are kept in an immature or semi-mature state and are therefore tolerogenic and suppress induction of immune responses. In addition, these DCs could down-regulate ongoing immune responses by attracting activated effector T-cells rendering them unresponsive. The mechanisms by which DC maturation is suppressed are largely unknown. It is also poorly
defined how this suppression can be mediated by cytokines and it is not clear whether a common "tolerance pathway" exists.

In the treatment of autoimmune diseases immunosuppressive or ablative therapies are most commonly used to eliminate autoreactive cells. However, in recent years, inhibiting the effector mechanisms such as cytokine blockade has been demonstrated to be effective in preventing immune activation in some diseases. In addition, new therapies are currently in clinical trials to target lymphoid cells more specifically, either by blocking a co-stimulatory signal needed for T or B cell activation, by eliminating the effector T cells or B cells, or by using autoantigen itself to induce tolerance. The induction of tolerance, thereby restoring normal regulatory mechanisms, has therefore additional potential as therapy for treatment of autoimmune diseases including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS) and type I diabetes. Tolerance prevents target organ damage, which remains an important therapeutic approach to autoimmune diseases. Furthermore, the increased risk of cancer and infection as well as toxicity by immunosuppressive drugs is avoided.

WO99/46281 relates to secreted and transmembrane polypeptides and their polynucleotides, useful for treating blood coagulation disorders, cancers and cellular adhesion disorders.

WO 03/080667 relates to nucleic acid encoding a TREM-4 or TREM-5 polypeptide, useful for treating e.g. inflammatory disorder, cancer, infertility or heart disease affecting microvascular compartments.

**SUMMARY OF THE INVENTION**

The present invention provides in one aspect, an isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, a variant, a splice variant, a fragment or a derivative thereof. In a further aspect, the invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence encoding said polypeptide according to the invention. In a further aspect, the invention relates to a vector containing the DNA of a nucleic acid molecule according to the invention. In yet a further aspect of the invention, it relates to a host cell comprising a vector according to the invention.

The invention provides in another aspect, a method for producing a polypeptide according to the invention comprising expressing the polypeptide encoded by a nucleic acid molecule according to the invention from a host cell and recovering the polypeptide. In a further aspect, the invention provides a method for preparing a cell or progeny thereof
capable of expressing a polypeptide according to the invention comprising transforming/transfecting a cell with a vector according to the invention.

In a further aspect, the invention provides an isolated CD300LG-ext polypeptide according to the invention for use in medicine, and in yet a further aspect a pharmaceutical composition comprising said polypeptide and a pharmaceutically acceptable carrier.

The invention relates in one aspect to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the induction of tolerogenic dendritic cells in a subject. In a further aspect, the invention provides the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of one or more disease or disorders selected form the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, astma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival.

The invention further provides the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament for the induction of tolerance of DC’s. The invention further provides the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the treatment or prevention of one or more diseases or disorders selected form the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, astma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and myasthenia gravis.

The invention further provides the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for reduced transcription or translation and thereby reduced expression of CD300LG protein in vivo after administration.

The invention further provides the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for the treatment or prevention of one or more diseases or disorders selected form the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, astma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other
dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and myasthenia gravis.

In another aspect, the invention provides an antibody which specifically binds to a polypeptide of the invention, or an antigen-binding fragment of said antibody.

In one aspect of the invention, an antibody or an antigen-binding fragment of said antibody, which inhibits DC-dependent allogenic T cell proliferation and/or inhibits DC-T cell clustering, is provided. In a further aspect of the invention, an antibody or an antigen-binding fragment of said antibody, which significantly inhibits DC-dependent allogenic T cell proliferation and/or inhibits DC-T cell clustering, is provided. In a further aspect, a pharmaceutical composition comprising the inhibitory antibody or a fragment thereof and a pharmaceutically acceptable carrier, is provided. In yet another aspect, the invention provides the use of the inhibitory antibody or a fragment thereof for the production of a medicament for the induction of tolerance of dendritic cells, and in yet a further aspect, the use of the inhibitory antibody or the fragment thereof for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival. In a further aspect, the invention provides the use of the inhibitory antibody or the fragment thereof for the preparation of a medicament for treating a subject having a disease or disorder associated with increased CD300LG full-length expression.

In another aspect, an antibody or an antigen-binding fragment of said antibody, which activates DC-dependent allogenic T cell proliferation and/or activates DC-T cell clustering, is provided. In yet another aspect, an antibody or an antigen-binding fragment of said antibody, which significantly activates DC-dependent allogenic T cell proliferation and/or activates DC-T cell clustering, is provided. In a further aspect, a pharmaceutical composition comprising the activating antibody or a fragment thereof and a pharmaceutically acceptable carrier, is provided. In yet another aspect, the invention provides the use of the activating antibody or a fragment thereof for the production of a medicament for the reduction of tolerance of dendritic cells, and in yet a further aspect, the use of the activating antibody or the fragment thereof for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting cancer or infections. In a further aspect, the invention provides the use of the activating antibody or a fragment
thereof for the preparation of a medicament for treating a subject having a disease or disorder associated with reduced CD300LG full-length expression.

In yet a further aspect of the invention, an assay method for in vitro determining the amount of CD300LG-FL in the tissues of a patient, preferably the method for determining tumor, autoimmune diseases, allergy, allograft survival, and infections, which comprises contacting a serum sample with an antibody or a fragment thereof according to the invention, is provided.

In yet a further aspect the invention provides variants and derivatives of CD300LG-ext that act as antagonists and/or agonists of the CD300LG receptor.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. IL-21, IL-10, TNF-α and TGF-β induces down-regulation of CD300LG mRNA levels in murine DCs. CD300LG transcript levels were measured by real time PCR analysis and normalized to expression of acidic ribosomal protein PO. Relative expression level indicates fold regulation by IL-21, IL-10, TNF-α or TGF-β compared to time-matched media controls and are presented as means±SEM (n=3). A t-test was used to determine if differences between control and cytokine-stimulated cells were statistically significant (*: p<0.05, **: p<0.01, ***: p<0.001).

Figure 2. Schematic illustration of comparison of amino acid sequences of human CD300LG-FL polypeptide splice variants. Schematic alignment of CD300LG-FL polypeptides, including CD300LG, TREMc and TREMB. Numbers of amino acids are indicated above each polypeptide, including signal peptide (sign pep), extracellular domain (ext domain), transmembrane domain (TM), and intracellular domain (int domain).

The term “signal sequence” as used herein refers to amino acid residues 1 to 18 of any of SEQ ID No. 4, 5, and 6.

The term “extracellular domain”, abbreviated ext, as used herein refers to amino acid residues 19 to 247 of SEQ ID No. 4 and amino acid residues 19 to 162 of SEQ ID No. 5, and 6.

The term “transmembrane domain”, abbreviated TM, as used herein refers to amino acid residues 248 to 269 of SEQ ID No. 4 and amino acid residues 163 to 184 of SEQ ID No. 5 and 6.

The term “intracellular domain” as used herein refers to amino acid residues 270 to 332 of SEQ ID No. 4, amino acid residues 185 to 233 of SEQ ID No. 5, and amino acid residues 185 to 222 of SEQ ID No. 6.
The term “CD300LG-FL polypeptides” as used herein refers to Full Length membrane bound polypeptides of SEQ ID Nos. 4, 5, 6 comprising extracellular domain, transmembrane domain, and intracellular domain.

The term “CD300LG-ext polypeptides” as used herein refers to polypeptides of SEQ ID No. 4, 5, and 6, comprising the extracellular domain, a variant, a splice variant, a fragment or a derivative thereof. In one aspect of the invention, the CD300LG-ext polypeptide is soluble.

The term “soluble” as used herein refers to polypeptides that during recombinant expression is secreted from the host cells and therefore are not membrane bound as the CE300LG-FL polypeptides.

The term “CD300LG polypeptides" as used herein refers to both CD300LG-FL and CD300LG-ext polypeptides.

The term “medicament” as used herein means a pharmaceutical composition suitable for administration of the pharmacologically active compounds to a patient.

The term “active compound” as used herein refers to CD300LG-ext polypeptide(s), either as the extracellular protein alone or the extracellular part fused to other proteins, antibodies specifically binding CD300LG-ext polypeptides, nucleic acid molecules encoding CD300LG-ext polypeptides, and nucleic acid agents inhibiting the expression of CD300LG polypeptides, which all induces and/or reduces the function of CD300LG-FL polypeptides.

The term “effective amount” as used herein means a dosage which is sufficient in order for the treatment of the patient to be effective compared with no treatment.

The term “effective amount” as used herein means a dosage which is sufficient in order for the treatment of the patient to be effective compared with no treatment.

The term “medicament” as used herein means a pharmaceutical composition suitable for administration of the pharmacologically active compounds to a patient.

The term “induction of tolerance” as used herein indicates that the active compound inhibits the interaction between CD300LG-FL polypeptides and their corresponding receptors/ligands and thereby their function.

The term “reduction of tolerance” as used herein indicates that the active compound activates CD300LG-FL polypeptides and their corresponding ligands/receptors and thereby their function.

The term “inhibiting the function of CD300LG-FL polypeptides” as used herein refers to decreased intracellular signaling as measured by inhibition of DC-dependent allogenic T cell proliferation as described in example 6 and 7, and/or inhibition of DC-T cell clustering as described in example 8, and/or inhibition of autoimmune diseases or disorders selected form
the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival. In one aspect of the invention, the inhibition is significant.

The term “activating the function of CD300LG-FL polypeptides” as used herein refers to increased intracellular signaling as measured by activation of DC-dependent allogenic T cell proliferation as described in example 6 and 7, and/or activation of DC-T cell clustering as described in example 8, and/or inhibition of diseases or disorders selected form the group consisting of infectious diseases and cancer. In one aspect of the invention, the activation and/or inhibition are significant.

The term “treatment of a disease or disorder” as used herein means the management and care of a patient having developed the disease, condition or disorder. The purpose of treatment is to combat the disease, condition or disorder. Treatment includes the administration of the active compounds to eliminate or control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

The term “prevention of a disease or disorder” as used herein is defined as the management and care of an individual at risk of developing the disease prior to the clinical onset of the disease. The purpose of prevention is to combat the development of the disease, condition or disorder, and includes the administration of the active compounds to prevent or delay the onset of the symptoms or complications and to prevent or delay the development of related diseases, conditions or disorders.

The term “pharmaceutical composition” as used herein means a product comprising an active compound optionally together with pharmaceutical excipients such as buffer, preservative and tonicity modifier, said pharmaceutical composition being useful for treating, preventing or reducing the severity of a disease or disorder by administration of said pharmaceutical composition to a person. Thus, a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term “pharmaceutically acceptable” as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term “buffer” as used herein refers to a chemical compound in a pharmaceutical composition that reduces the tendency of pH of the composition to change over time as would otherwise occur due to chemical reactions. Buffers include chemicals such as sodium phosphate, TRIS, glycine and sodium citrate.
The term "preservative" as used herein refers to a chemical compound which is added to a pharmaceutical composition to prevent or delay microbial activity (growth and metabolism). Examples of pharmaceutically acceptable preservatives are phenol, m-cresol and a mixture of phenol and m-cresol.

The term "isotonicity agent" as used herein refers to a chemical compound in a pharmaceutical composition that serves to modify the osmotic pressure of the pharmaceutical composition so that the osmotic pressure becomes closer to that of human plasma. Isotonicity agents include NaCl, glycerol, mannitol etc.

The term "stabiliser" as used herein refers to chemicals added to peptide containing pharmaceutical compositions in order to stabilise the peptide, i.e. to increase the shelf life and/or in-ude time of such compositions. Examples of stabilisers used in pharmaceutical compositions are L-glycine, L-histidine, arginine, polyethylene glycol, and carboxymethylcellulose.

The term "surfactant" as used herein refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, the head, and a fat-soluble (lipophilic) segment. Surfactants accumulate preferably at interfaces, which the hydrophilic part is orientated towards the water (hydrophilic phase) and the lipophilic part towards the oil- or hydrophobic phase (i.e. glass, air, oil etc.). The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. Furthermore, surfactants lower the surface tension of a liquid. Surfactants are also known as amphipathic compounds. The term "Detergent" is a synonym used for surfactants in general.

DESCRIPTION OF THE INVENTION

Tolerogenic DCs are important to suppress overwhelming and unwanted activation of the immune system and to maintain the normal homeostasis of the entire organism. Four cytokines, including IL-21, IL-10, TNF-α and TGF-β have been described to induce or sustain tolerogenic DCs. The mechanisms by which DC maturation is suppressed by these cytokines are largely unknown and it is not clear whether a common "tolerance pathway" exists.

Using cDNA microarray analyses, the present invention relates to the identification of CD300 antigen like family member G (CD300LG) mRNA as down-regulated in murine dendritic cells (DCs) by the "tolerogenic" cytokines IL-21, IL-10, TNF-α and TGF-β. Down-regulation of CD300LG protein expression and thereby CD300LG function therefore has the potential of being essential for induction of tolerance and to maintain the normal homeostasis of the entire organism.
In mouse, CD300LG encodes a protein, which is part of a multigene family containing a single Ig domain and expressed mainly in cells of the myeloid lineage (Chung et al., 2003, 171: 6541-6548). In humans, homologs of murine CD300LG polypeptides exists, and alternatively spliced form of the same transcript include “triggering receptor expressed on myeloid cells” or TREM, including TREM-4α and TREM-4β (WO 99/46281, WO 03/080667) (see fig. 2). Collectively the CD300LG splice variants are referred to as CD300LG Full Length (CD300LG-FL) polypeptides. TREM-4 (α and β) are strongly expressed in the capillaries of subcutaneous adipose tissue, lymph nodes, thymus, as well as in liver sinusoid endothelium cells, but not in the capillaries of demis, lung and placenta, the endothelium of arteries, arterioles, veins, venules and lymphatic vessels, and TREM-4 have been suggested to be involved in the regulation of inflammation, neoplastic transformation, myeloid cells, atherosclerosis, tumorigenesis, spermatogenesis, and microcirculation (WO 03/080667).

CD300LG-FL polypeptides are potential type I receptors, consisting of a signal peptide, an extracellular domain, a transmembrane domain, and an intracellular domain (Figure 2). The murine protein contains a single tyrosine residue in the intracellular domain; however, this is not positioned in a consensus motif for binding by any common signaling proteins. Furthermore, the tyrosine is not conserved in human splice variants and finally, no charged lysine residue in the transmembrane region, which is reminiscent of that of activating NK and myeloid cell receptors that pair with DAP12. Therefore, whether CD300LG-FL polypeptides can be regarded as a receptor, resulting in intracellular signaling in DCs, or as a ligand interacting with receptors expressed on other cells, thereby resulting in intracellular signaling in these cells, is not clear. However, agents that inhibit the interaction between membrane-bound CD300LG (CD300LG-FL polypeptides) and corresponding CD300LG-receptors/ligands can potentially inhibit the function of CD300LG-FL polypeptide function and thereby induce tolerance. This can be used to prevent and/or treat disorders or diseases related to the function of CD300LG such as autoimmunity, allergic hypersensitivity, and/or allograft rejection.

Therefore, active medicaments and/or compounds that induces tolerance, according to an aspect of the present invention, may be one or more of:

1) soluble CD300LG proteins expressing the extracellular parts of CD300LG-FL polypeptides, referred to as CD300LG-ext polypeptides, either as the extracellular protein alone or the extracellular part fused to other proteins, 2) antagonistic monoclonal antibodies or monoclonal antibody fragments that bind to CD300LG-ext polypeptides, thereby inhibiting the interaction
between membrane-bound CD300LG-FL polypeptides and their corresponding receptors/ligands, or

3) agents preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational or post-translational level.

Alternatively, agents that activate the function of CD300LG-FL polypeptides may be involved in the reduction of tolerance, and can be used to prevent and/or treat infections and/or cancer. Therefore, active medicaments that reduce tolerance, according to the present invention, may be agonistic monoclonal antibodies or monoclonal antibody fragments that specifically bind to CD300LG-ext polypeptides, thereby activating the function of CD300LG-FL polypeptides.

The invention encompasses methods for preparation of active compounds inhibiting the function of CD300LG-FL polypeptides, including CD300LG-ext polypeptides, which are polypeptides comprising the extracellular part of CD300LG-FL polypeptides, agonistic or antagonistic antibodies against CD300LG-ext polypeptides, and agents preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational or post-translational level.

In addition, the invention encompasses methods for testing agents activating/inhibiting the function of CD300LG-FL polypeptides and thereby their potential for treating syndromes associated with C300LG-FL polypeptide dys-regulation.

Nucleic acid sequences encoding human members of the CD300LG family includes at least sequences provided in SEQ ID No. 1-3 (Genbank accession Nos. BC025395, AF427619, and AF42762 respectively, and corresponding amino acids sequences provided in SEQ ID No. 4-6 (Genbank accession Nos, AAH25395, AAN86134, and AAN86135 respectively).

Using a PCR based strategy the extracellular domains of human and murine CD300LG-FL polypeptides, herein referred to as CD300LG-ext polypeptides, can be amplified by PCR from reverse transcribed cDNA from human and mouse total RNA from a combination of total RNA from a variety of tissues from human and mouse, respectively.

In one aspect of the invention, an isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, a variant, or a splice variant thereof, is provided. In one aspect of the invention, the CD300LG-ext polypeptide is a fragment of such a CD300LG-ext polypeptide.
In one aspect of the invention, the CD300LG-ext polypeptide is a derivative of such a CD300LG-ext polypeptide.

In one aspect of the invention, an isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, is provided.

In one aspect of the invention, the CD300LG-ext polypeptide is a splice variant of the CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, such as a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

In another aspect of the invention, an isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6, a variant, a splice variant, a fragment or derivative thereof, is thus provided. In one aspect of the invention, the CD300LG-ext polypeptide is a fragment of such a CD300LG-ext polypeptide.

In one aspect of the invention, the CD300LG-ext polypeptide is a derivative of such a CD300LG-ext polypeptide.

In a further aspect of the invention, an isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6, is provided.

In one aspect of the invention, the CD300LG-ext polypeptide is a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 or a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6. In a further aspect of the invention, said variant is selected from the group consisting of a variant of a CD300LG-ext polypeptide having the amino acid sequence corresponding to amino acids 19-247 of SEQ ID No. 4, and a variant of CD300LG-ext polypeptide having the amino acid sequence corresponding to amino acids 19-162 of SEQ ID No. 5 or 6.

The term "variant" as used herein refers either to a naturally occurring variation of a given peptide or a recombinantly prepared or otherwise modified variation of a given peptide or protein in which one or more amino acid residues have been modified by amino acid substitution, addition, deletion, insertion or inversion.

A variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 or a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 may comprise such substitution(s), deletion(s), addition(s) (at the N- and/or C-terminal), and/or insertion(s) (between the N- and the C-terminal) at any position or combination of positions.

In one aspect of the invention, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 or a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.
polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions and/or deletions of amino acid residues at the N-terminal and/or C-terminal of the polypeptide. It is understood, that while amino acid residues are added and/or deleted at the N- and/or C-terminal, such a variant may also comprise deletions, substitutions and/or inversions in the region between the N- and the C-terminal.

In one aspect of the invention, a variant CD300LG-ext polypeptide of the invention having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, a fragment or derivative thereof, further comprises one or more of the following:

(i) one or more amino acid residues derived from the signal sequence of amino acids 1-18 of SEQ ID No. 4 at the N-terminus of the CD300LG-ext polypeptide. Such amino acid may for instance be amino acid residue 18 (A) at the N-terminus of a sequence of amino acids 19-247 of SEQ ID No. 4,

(ii) one or more amino acid residues derived from the transmembrane domain at the C-terminus of the CD300LG-ext polypeptide. Such amino acids may for instance be amino acid residues 248-251 (ILAP) of SEQ ID No. 4,

(iii) functional sequences attached to its N-terminus, or

(iv) functional sequences attached to its C-terminus.

In one aspect of the invention, a variant CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6, a fragment or derivative thereof further comprises one or more of the following:

(i) one or more amino acid residues derived from the signal sequence of amino acids 1-18 of SEQ ID No. 5 or 6 at the N-terminus of the CD300LG-ext polypeptide. Such amino acid may for instance be amino acid residue 18 (A) at the N-terminus of a sequence of amino acids 19-162 of SEQ ID No. 5 or 6,

(ii) one or more amino acid residues derived from the transmembrane domain at its C-terminus of the CD300LG-ext polypeptide. Such amino acids may for instance be amino acid residues 163-166 (ILAP) of sequence ID NO 5 or 6,

(iii) functional sequences attached to its N-terminus, or

(iv) functional sequences attached to its C-terminus.

The term "functional sequences" as used in paragraphs (iii) and (iv) above is intended to indicate sequences of amino acid residues, which impart a function to the polypeptide, wherein said function is unrelated to the function of the CD300LG-ext
polypeptide of the invention. Such “functional sequences” or “tags” could for instance aid in the purification, localization and/or identification of the polypeptides.

In one aspect of the invention, the CD300LG-ext polypeptide variant has an identity of at least 90%, such as at least 95%, for instance at least 98% to the amino acid sequence of SEQ ID No. 4, 5 or 6. In one aspect, the CD300LG-ext polypeptide variant has an identity of at least 80%, such as at least 85%, for instance at least 90%, such as at least 95%, for instance at least 98% at the DNA level of amino acid sequence of SEQ ID No. 4, 5 or 6.

In one aspect of the invention, the CD300LG-ext polypeptide variant has an similarity of at least 90%, such as at least 95%, for instance at least 98% to the amino acid sequence of SEQ ID No. 4, 5 or 6. In one aspect, the CD300LG-ext polypeptide variant has an similarity of at least 80%, such as at least 85%, for instance at least 90%, such as at least 95%, for instance at least 98% at the DNA level of amino acid sequence of SEQ ID No. 4, 5 or 6.

In one aspect of the invention, the CD300LG-ext polypeptide has at least 90% identity to the amino acid sequence of amino acids 19-247 of SEQ ID No. 4.

In a further aspect of the invention, the CD300LG-ext polypeptide has at least 95% identity to the amino acid sequence of amino acids 19-247 of SEQ ID No. 4.

In a further aspect of the invention, the CD300LG-ext polypeptide has at least 98% identity to the amino acid sequence of amino acids 19-247 of SEQ ID No. 4.

In a another aspect of the invention, the CD300LG-ext polypeptide has at least 90% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

In a further aspect of the invention, the CD300LG-ext polypeptide has at least 95% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

In a further aspect of the invention, the CD300LG-ext polypeptide has at least 98% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or between polypeptides, as the case may be, as determined by the number of matches between strings of two or more nucleotide residues or two or more amino acid residues. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a sequence relationship that includes both identical matches and conservative substitution
matches. If two polypeptide sequences have, for example, (fraction (10/20)) identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If, in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% ((fraction (15/20))). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

Conservative modifications to the CD300LG-ext polypeptides according to the invention (and the corresponding modifications to the encoding nucleotides) will produce CD300LG-ext polypeptides having functional and chemical characteristics similar to those of naturally occurring CD300LG-ext polypeptide. In contrast, substantial modifications in the functional and/or chemical characteristics of CD300LG-ext polypeptides according to the invention may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example, MacLennan et al., 1998, Acta Physiol. Scand. Suppl. 643:55-67; Sasaki et al., 1998, Adv. Biophys. 35:1-24, which discuss alanine scanning mutagenesis).

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the CD300LG-ext polypeptides according to the invention, or to increase or decrease the affinity of the CD300LG-ext polypeptides described herein.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human CD30LG-ext polypeptides that are homologous with non-human CD30LG-ext polypeptides, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0±1); glutamate (+3.0±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5±1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.5); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions.".
Variant CD300LG-ext polypeptides of the present invention may also include non-naturally occurring amino acids.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 248 to 332 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 248 to 269 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 270 to 332 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 163-233 of SEQ ID No. 5.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance
more than 80% identical to an amino acid sequence consisting of amino acid residues 163-222 of SEQ ID No. 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 163 to 184 of SEQ ID No. 5 and 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 185 to 233 of SEQ ID No. 5.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% identical, such as more than 95% identical, for instance more than 90% identical, such as more than 85% identical, for instance more than 80% identical to an amino acid sequence consisting of amino acid residues 185 to 222 of SEQ ID No. 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 248 to 332 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80%
similar to an amino acid sequence consisting of amino acid residues 248 to 269 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 270 to 332 of SEQ ID No. 4.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 163-233 of SEQ ID No. 5.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 163-222 of SEQ ID No. 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 163 to 184 of SEQ ID No. 5 and 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80%
similar to an amino acid sequence consisting of amino acid residues 185 to 233 of SEQ ID No. 5.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises additions of amino acid residues in the C-terminal, wherein said C-terminally added amino acids does not comprise an amino acid sequence, which is more than 98% similar, such as more than 95% similar, for instance more than 90% similar, such as more than 85% similar, for instance more than 80% similar to an amino acid sequence consisting of amino acid residues 185 to 222 of SEQ ID No. 6.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 or a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises no more than 50, such as no more than 45, for instance no more than 40, such as no more than 35, for instance no more than 30, such as no more than 25, for instance no more than 20, such as no more than 15, for instance no more than 10, such as no more than 5, for instance no more than 4, such as no more than 3, for instance no more than 2, such as no more than 1, for instance no additions of amino acid residues in the C-terminal.

In one embodiment, a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4 or a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6 comprises less than 20 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 19 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 18 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 17 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 16 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 15 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 14 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 13 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 12 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 11 modifications (substitutions, deletions,
additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 10 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 9 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 8 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 7 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 6 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment a variant comprises less than 5 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment, a variant comprises less than 4 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment, a variant comprises less than 3 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment, a variant comprises less than 2 modifications (substitutions, deletions, additions, insertions) relative to the native peptide. In one embodiment, a variant comprises only a single modification (substitutions, deletions, additions, insertions) relative to the native peptide.

A skilled artisan will be able to determine suitable variants of the CD300LG-ext polypeptides as set forth in SEQ ID Nos. 4, 5 or 6 using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a CD300LG-ext polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a CD300LG-ext polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of the CD300LG-ext polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a
comparison, one can predict the importance of amino acid residues in a CD300LG-ext polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of CD300LG-ext polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a CD300LG-ext polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays as described herein. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou et al., Biochemistry, 13(2):222-245 (1974); Chou et al., Biochemistry, 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.


Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res., 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NIH Bethesda, Md. 20894; Altschul et al., supra).

The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, Wis.), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3 times the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually (fraction (1/10)) times the gap opening penalty), as well
as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the
algorithm. A standard comparison matrix (see Dayhoff et al., Atlas of Protein Sequence and
Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff et al., Proc.
Natl. Acad. Sci USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also
used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:
Algorithm: Needleman et al., J. Mol. Biol, 48:443-453 (1970); Comparison matrix:
Penalty: 12, Gap Length Penalty: 4, Threshold of Similarity: 0.

The GAP program is useful with the above parameters. The aforementioned
parameters are the default parameters for polypeptide comparisons (along with no penalty
for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the
following: Algorithm: Needleman et al., J. Mol Biol., 48:443-453 (1970); Comparison matrix:
matches=+10, mismatch=0, Gap Penalty: 50, Gap Length Penalty: 3.

The GAP program is also useful with the above parameters. The aforementioned
parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties,
comparison matrices, thresholds of similarity, etc. may be used, including those set forth in
choices to be made will be apparent to those of skill in the art and will depend on the specific
comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and
additionally, whether the comparison is between given pairs of sequences (in which case
GAP or BestFit are generally preferred) or between one sequence and a large database of
sequences (in which case FASTA or BLASTA are preferred).

In the present context the three-letter or one-letter indications of the amino acids
have been used in their conventional meaning as indicated in table 1. Unless indicated
explicitly, the amino acids mentioned herein are L-amino acids. Further, the left and right
ends of an amino acid sequence of a peptide are, respectively, the N- and C-termini unless
otherwise specified.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter code</th>
<th>One-letter code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>P</td>
</tr>
</tbody>
</table>

Table 1: Abbreviations for amino acids:
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter code</th>
<th>One-letter code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>A</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>V</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>I</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>F</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>W</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glu</td>
<td>E</td>
</tr>
<tr>
<td>Aspartic Acid</td>
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<td>D</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>T</td>
</tr>
</tbody>
</table>

In one aspect of the invention, a CD300LG-ext polypeptide of the invention as described above (i.e. including variants, splice variants, fragments and derivatives) inhibits DC-dependent allogenic T cell proliferation as described in example 6 and 7 and/or inhibits DC-T cell clustering as described in example 8. Inhibition by CD300LG-ext polypeptides is defined as production of a measurable statistical change of measured parameters by CD300LG-ext treatment compared to controls.

In one aspect of the invention, the CD300LG-ext polypeptide according to the invention is soluble, soluble referring to polypeptides being secreted from host cells during recombinant expression, in contrast to CD300LG-FL polypeptides that are membrane bound.

In one aspect of the invention, an isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide according to the invention, is provided.

In one aspect of the invention, the nucleic acid molecule is DNA.

In another aspect of the invention, the nucleic acid molecule is RNA.

In a further aspect of the invention, a nucleic acid molecule having a nucleotide sequence of 42-782 of SEQ ID No. 1, is provided.
In a further aspect of the invention, a nucleic acid molecule having a nucleotide sequence of 96-782 of SEQ ID No. 1, is provided.

In a further aspect of the invention, a nucleic acid molecule having a nucleotide sequence of 62-547 of SEQ ID No. 2, is provided.

In a further aspect of the invention, a nucleic acid molecule having a nucleotide sequence of 116-547 SEQ ID No. 2, is provided.

Once a nucleic acid encoding a member of CD300LG-ext polypeptides has been cloned, the extracellular domain can then be expressed recombinantly using techniques as described herein.

For example, a nucleic acid encoding a CD300LG-ext polypeptide can be produced, inserted into a vector and transformed/transfected into a prokaryotic or eukaryotic host cell, respectively using well known techniques described herein and further known in the art (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York).

The term “vector” refers to a plasmid, virus or other vehicle known in the art that can be manipulated by insertion or incorporation of a polynucleotide. Such vectors can be used for genetic manipulation or can be used to transcribe or translate the inserted polynucleotide. A vector generally contains at least an origin of replication for propagation in a cell and a promoter.

By “promoter” is meant a minimal sequence sufficient to direct transcription. Both constitutive and inducible promoters are activated by external signals or agents.

In one aspect of the invention, a vector containing herein described DNA, is provided.

In a further aspect of the invention, a host cell comprising a vector is provided.

In one aspect of the invention, a host cell comprising the nucleic acid molecule as described herein operably linked to a heterologous promoter, is provided. In a further aspect of the invention, the host cell is a prokaryotic cell. In yet a further aspect of the invention, the host cell is a eukaryotic cell, such as a mammalian cell.

In one aspect of the invention, a method for producing a polypeptide according to the invention comprising expressing the polypeptide encoded by the nucleic acid molecule from a host cell and recovering the polypeptide, is provided.

In a further aspect of the invention, a method for preparing a cell or progeny thereof capable of expressing a polypeptide according to the invention comprising transforming/transfecting a cell with the vector, is provided.

As used herein, the terms “transformation” and “transfection” means a genetic change in a cell following incorporation of DNA exogenous to the cell. This, a
“transformed/transfected cell” is a cell into which a DNA molecule has been introduced by means of recombinant DNA techniques.

Transformation/ transfection of a host cell with DNA may be carried out by conventional techniques known to those skilled in the art. For example, when the host is a eukaryote, methods of DNA transfection include, for example, calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, and viral vectors.

When cloning in bacterial systems, expression vectors will comprise a promoter capable of directing the transcription of a cloned gene or cDNA. The promoter may be any DNA sequence that exhibits transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of promoters are constitutive promoters such as T7 and the like.

When cloning CD300LG-ext polypeptides in mammalian cell systems, expression vectors will comprise a promoter capable of directing the transcription of a cloned gene or cDNA. The promoter may be any DNA sequence that exhibits transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of promoters are constitutive promoters such as SV40, RSV, CMV, and the inducible promoters derived from the genome of mammalian cells (e.g. methallothionein promoter) or from mammalian viruses (e.g. the mouse mammary tumor virus long terminal repeat; the adenovirus promoter). Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequence of the invention.

To direct the CD300LG-ext polypeptide of the present invention into the secretory pathway of the mammalian host cells, a secretory signal sequence (also known as a leader sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequences encoding the human CD300LG-ext polypeptide in the correct reading frame. Secretory signal sequences are commonly positioned 5’ to the DNA sequence encoding the peptide. The secretory signal sequence may be that normally associated with the protein, may be from a gene encoding another secreted protein, or may be a synthetic peptide.

For CD300LG-ext cloning for expression in yeast, expression vectors will comprise a promoter capable of directing the transcription of a cloned gene or cDNA. The promoter may be any DNA sequence that exhibits transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

A number of vectors containing constitutive or inducible promoters may be used.
Alternatively, vectors that facilitate integration of foreign nucleic acid sequences into a yeast chromosome, via homologous recombination are known in the art.

For secretion from yeast cells, suitable signal peptides include, without limitation the α-factor signal peptide (cf. US 4,870,008), the signal peptide of mouse salivary amylase (cf. O. Hagenbuchle et al., *Nature* 289, 1981, pp. 643-646), a modified carboxypeptidase signal peptide (cf. L.A. Valls et al., *Cell* 48, 1987, pp. 887-897), the yeast BAR1 signal peptide (cf. WO 87/02670), or the yeast aspartic protease 3 (YAP3) signal peptide (cf. M. Egel-Mitani et al., *Yeast* 6, 1990, pp. 127-137). Furthermore, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and upstream of the DNA sequence encoding the human Factor VII variants. The function of the leader peptide is to allow the expressed peptide to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the culture medium (i.e. exportation of the human Factor VII polypeptide variants across the cell wall or at least through the cellular membrane into the periplasmic space of the yeast cell). In one embodiment, the leader peptide is the yeast alpha-factor leader (the use of which is described in e.g. US 4,546,082, US 4,870,008, EP 16 201, EP 123 294, EP 123 544 and EP 163 529). Alternatively, the leader peptide may be a synthetic leader peptide, i.e., a leader peptide not found in nature. Synthetic leader peptides may, for instance, be constructed as described in WO 89/02463 or WO 92/11378.

The host cells used for expression of CD300LG-ext polypeptides may be vertebrate, insect, fungal, or bacterial cells. The CD300LG-ext polypeptide variants may also be produced in transgenic animals or plants.

For producing CD300LG-ext polypeptides for testing the CD300LG constructs can be produced in mammalian cells, more specifically CD300LG-ext polypeptides in HEK293 6E cells will be expressed using a transient expression system. Alternatively, CD300LG-ext polypeptides for testing can be produced in bacteria.

For long-term expression of CD300LG-ext polypeptides in host cells, stable expression is preferred. Examples of mammalian cell lines for use in the present invention are the COS-1 (ATCC CRL 1650), baby hamster kidney (BHK) and 293 (ATCC CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) cell lines. A preferred BHK cell line is the tk-ts13 BHK cell line (Waechter and Baserga, *Proc. Natl. Acad. Sci. USA* 79:1106-1110, 1982, incorporated herein by reference), hereinafter referred to as BHK 570 cells. The BHK 570 cell line has been deposited with the American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852, under ATCC accession number CRL 10314. A tk-ts13 BHK cell line is also available from the ATCC under accession number CRL 1632. In addition, a number of
other cell lines may be used within the present invention, including Rat Hep I (Rat hepatoma; ATCC CRL 1600), Rat Hep II (Rat hepatoma; ATCC CRL 1548), TCMK (ATCC CCL 139), Human lung (ATCC HB 8065), NCTC 1469 (ATCC CCL 9.1), CHO (ATCC CCL 61) and DUKX cells (Urlaub and Chasin, Proc. Natl. Acad. Sci. USA 77:4216-4220, 1980).

Examples of suitable yeasts cells include cells of Saccharomyces spp. or Schizosaccharomyces spp., in particular strains of Saccharomyces cerevisiae or Saccharomyces kluveri. Methods for transforming yeast cells with heterologous DNA and producing heterologous polypeptides there from are described, e.g. in US 4,599,311, US 4,931,373, US 4,870,008, 5,037,743, and US 4,845,075, all of which are hereby incorporated by reference. Transformed cells are typically selected by a phenotype determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient, e.g. leucine. A preferred vector for use in yeast is the POT1 vector disclosed in US 4,931,373. The DNA sequences encoding Factor VII variants may be preceded by a signal sequence and optionally a leader sequence, e.g. as described above.

Further examples of suitable yeast cells are strains of Kluveromyces, such as K. lactis, Hansenula, e.g. H. polymorpha, or Pichia, e.g. P. pastoris (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279).

Examples of other fungal cells are cells of filamentous fungi, e.g. Aspergillus spp., Neurospora spp., Fusarium spp. or Trichoderma spp., in particular strains of A. oryzae, A. nidulans or A. niger. The use of Aspergillus spp. for the expression of proteins is described in, e.g., EP 272 277, EP 238 023, EP 184 438 The transformation of F. oxysporum may, for instance, be carried out as described by Malardier et al., 1989, Gene 78: 147-156. The transformation of Trichoderma spp. may be performed for instance as described in EP 244 234.

Following transformation/transfection of a host cell and expression of CD300LG-ext polypeptides, CD300LG-ext polypeptides should be further purified. Purification may be achieved using any method known in the art, including size exclusion chromatography, ion-exchange chromatography, affinity chromatography, electrophoretic procedures (e.g. preparative isoelectric focusin (IEF), and/or differential solubility (e.g. ammonium sulfate precipitation). See, generally, Scopes, Protein Purification, Springer-Verlag, New York, 1982; and Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989.

The CD300LG-ext polypeptides of the present invention also include derivatives of CD300LG-ext polypeptides as mentioned above. The term "derivative" as used herein refers to a peptide or protein or variant or fragment thereof that are modified, i. e., by covalent
attachment of any type of molecule, preferably having bioactivity, to the parent polypeptide. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters, PEGylations and the like.

In one aspect of the invention, the derivatives remain soluble as defined above and are in a further aspect of the invention capable of causing an effect comparable to CD300LG-ext polypeptides in assays described in example 6, 7, and/or 8, i.e. a statistical change of measured parameters compared to controls.

According to the invention, derivatives of CD300LG-ext polypeptides include polypeptides in which one or more of the amino acids therein has an altered side chain. Such derivatized polypeptides include, for example, those comprising amino acids in which free amino groups form amine hydrochlorides, p-toluene sulfonyl groups, carobenzoxyl groups; the free carboxyl groups form salts, methyl and ethyl esters; free hydroxyl groups that form 0-acyl or 0-alkyl derivatives as well as naturally occurring amino acid derivatives, for example, 4- hydroxyproline, for proline, 5-hydroxylsine for lysine, homoserine for serine, ornithine for lysine etc. Also included are amino acid derivatives that can alter covalent bonding, for example, the disulfide linkage that forms between two cysteine residues that produces a cyclized polypeptide.

CD300LG-ext derivatives can have an altered glycosylation pattern or can be non-glycosylated.

The present invention also provides fusion proteins comprising a CD300LG-ext polypeptide of the invention covalently attached to a non-CD300LG-ext polypeptide, for instance a cytotoxic polypeptide or a polypeptide selected so as to confer stability of the fusion protein in plasma or to enhance bioavailability. Examples of the non-CD300LG-ext polypeptide are human serum albumin (HSA), HSA fragments, IgG1, and Fc portion of IgG1.

In one aspect of the invention, an antibody which specifically binds to CD300LG-ext polypeptides as defined herein, or an antigen-binding fragment of said antibody, is provided. An antibody "specifically binding to" a CD300LG-ext polypeptide to" is intended to indicate that the antibody recognize an epitope within a CD300LG-ext polypeptide, while only having little or no detectable reactivity with other portions of CD300LG-FL, particularly the transmembrane domain and the intracellular domain. Typically, the antibody binds with an affinity corresponding to a $K_d$ of about $10^{-7}$ M or less, such as about $10^{-8}$ M or less, such as about $10^{-9}$ M or less, about $10^{-10}$ M or less, or about $10^{-11}$ M or even less when determined by for instance surface plasmon resonance (SPR) technology in a BIACore 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a $K_d$ that is at least ten-fold lower,
such as at least 100 fold lower, for instance at least 1000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen.

In one aspect of the invention, the antibodies specifically binds soluble CD300LG-ext polypeptides or fragments thereof and can be used for various diagnostic and therapeutic purposes.

The term "antibody" is intended to indicate an immunoglobulin molecule or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions for significant periods of time such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen). The term antibody also includes diabodies and single chain antibodies.

Furthermore, although the two domains of the Fv fragment, V\(_L\) and V\(_H\), are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single peptide chain in which the V\(_L\) and V\(_H\) regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Other forms of single chain antibodies, such as diabodies are included within the term antibody.

It should also be understood that the term antibody also generally includes polyclonal antibodies, monoclonal antibodies (mAbs), antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, and anti-idiotypic (anti-Id) antibodies to antibodies. An antibody as generated can possess any isotype.

The term "immunoglobulin" refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as V\(_H\)) and a heavy chain constant region. The heavy chain constant region typically is comprised of three domains, C\(_H\)1, C\(_H\)2, and C\(_H\)3. Each light chain typically is comprised of a light chain variable region...
(abbreviated herein as $V_L$) and a light chain constant region. The light chain constant region typically is comprised of one domain, $C_L$. The $V_{H}$ and $V_L$ regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each $V_{H}$ and $V_L$ is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901-917 (1987)).

The term "fragment of an antibody" or "antibody-fragment" is intended to indicate a fragment of an antibody which fragment retains the ability to specifically bind to an antigen. Examples of binding fragments encompassed within the term "antibody" include (i) a Fab fragment, a monovalent fragment consisting of the $V_{L}$, $V_{H}$, $C_L$ and $C_{H1}$ domains; (ii) $F(ab)_2$ and $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the $V_{H}$ and $C_{H1}$ domains; (iv) a Fv fragment consisting essentially of the $V_{L}$ and $V_{H}$ domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature 341, 544-546 (1989)), which consists essentially of a $V_{H}$ domain; and (vi) an isolated complementarity determining region (CDR).

The antibody fragments may be provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques.

The antibodies of the present invention encompasses monoclonal antibodies, polyclonal antibodies, bispecific antibodies, Fab antibody fragments, $F(ab)_2$ antibody fragments, Fv antibody fragments (e.g., $V_{H}$ or $V_L$), single chain Fv antibody fragments and dsFv antibody fragments. Furthermore, the antibody molecules of the invention may be fully human antibodies, humanized antibodies, or chimeric antibodies. In some embodiments, the antibody molecules are monoclonal, fully human antibodies. Monoclonal antibodies encompass antibodies obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Monoclonal antibodies are advantageous in that they may be synthesized by a hybridoma culture, essentially uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being amongst a substantially homogeneous population of antibodies, and is not to be constructed as required production of the antibody by any particular method.

In one aspect of the invention, the antibody is a monoclonal antibody.
In a further aspect of the invention, the antibody is a human antibody, a humanized antibody, or chimeric antibody.

In yet a further aspect of the invention, the antibody inhibits DC-dependent allogenic T cell proliferation as described in assays of example 6 and 7 and/or inhibits DC-T cell clustering as described in assay of example 8.

Polyclonal antibodies to an antigen of interest can be produced by various procedures well known in the art. For example, an antigen derived from CD300LG-ext polypeptides according to the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc., to induce the production of antisera containing polyclonal antibodies specific for CD300LG-ext polypeptides. Various adjuvants may be used to increase the immunological response, depending on the host species, and include, but are not limited to, Freund's (complete and incomplete) adjuvant, mineral gels, such as aluminium hydroxide, and surface active substances, such as lyssolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol.

Monoclonal antibodies may be made using the hybridomas method first described by Kohler et al., Nature, 256: 495, 1975, or by other well-known, subsequently developed methods, including, but not limited to, phage display technologies. In the hybridoma method, a mouse or appropriate host animal is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes are then fused with myeloma cells using a suitable fusing agent to form hybridoma cells. The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. The culture medium in which the hybridoma cells are grown are assayed for production of monoclonal antibodies directed against the CD300LG-ext polypeptides according to the invention. Once identified, clones may be subcloned by limited dilution procedure and grown by standard methods. The nucleotide sequence encoding a monoclonal antibody or antibody fragments is readily isolated and sequenced using conventional procedures and can be done by those skilled in the art. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cell. Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, including, but not limited to, recombinant DNA techniques, site directed mutagenesis, and PCR, to generate antibodies
having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies or any portion of the antibodies which may enhance or reduce biological activities of the antibodies. Fragments (or analogs) of antibodies can be readily prepared by those of ordinary skill in the art.

For some uses, including therapeutic use of antibodies, it may be preferable to use chimeric, humanized or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art (e.g. Morrison, Science, 229: 1202, 1985; Oi et al., Biotechniques, 4: 214 1986; U.S. Patent No. 5,807,715; 4,816,567, and 4,816,397.

Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve antigen binding. These framework substitutions are identified by methods well known in the art, e.g. by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (Queen et al., U.S. Patent No 5,585,089; Riechmann et al., Nature 332:323, 1988). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,595; Padlan, Molecular Immunology, 28(4/5):489-498, 1991; Studnicka et al, Protein Engineering 7(6):805-814, 1994; Roguska et al., Proc. Natl. Acad. Sci. USA, 91:969-973, 1994), and chain shuffling (U.S. Patent No 5,565,332).

Human antibodies can be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulin, but which can express human immunoglobulin genes (Lonberg and Huszar, Int. Rev. Immunol. 13:65-93, 1995; PCT publications WO 98/24893; WO92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S: Patent NOs. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771, and 5,939,598. In addition, companies such as Abgenix Inc. (Freemont, CA), Medarex (NJ), and Genpharm (San Jose, CA) can be engaged
to provide human antibodies directed against a selected antigen using technology similar to that described above.

The binding specificity of antibodies can be determined by immunoprecipitation or by in vitro binding assays, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) or by immunofluorescence and flow cytometry or by Western blot analyses.

Once an antibody molecule of the invention has been produced by any method described above, it may then be purified by any method known in the art for purification of an immunoglobulin molecule, for example by chromatography (e.g. ion exchange, affinity, and gel filtration), centrifugation, differential solubility, or by other standard techniques for the purification of proteins.

In one embodiment, antibodies of the invention provides methods for detecting the presence, activity, or expression of the CD300LG polypeptides of the invention in a biological material, such as cells, blood, saliva, urine, biopsied tissue, and so forth. The increased or decreased presence of the CD300LG polypeptides in a sample relative to control sample can be determined by contacting the biological material with an antibody which can detect directly or indirectly the presence or expression of soluble or membrane bound CD300LG polypeptides.

In another embodiment, antibodies of the invention acts as antagonists of CD300LG-FL polypeptides and may be used as a therapeutic agent for inducing tolerogenic DCs and thereby reducing systemic and/or local inflammatory responses. Such antibodies can block the binding of CD300LG-FL polypeptides to their corresponding receptors/ligands and thereby prevent subsequent signal transduction and inflammatory responses.

In yet another embodiment, antibodies of the invention acts as agonist of CD300LG-FL polypeptides and/or ligands/receptors and may be used as a therapeutic agent for inhibiting the formation of tolerogenic DCs and thereby be used for the induction of an immunological response in infectious diseases and cancer.

In one embodiment, the nucleic acid sequences encoding the CD300LG-ext polypeptides are directly administered in vivo, where they are expressed to produce the encoded products. Preferably, the nucleic acid molecules include at least nucleic acid residues 42 to 782 of SEQ ID No. 1, encoding amino acid residues 1 to 247 of SEQ ID No. 4 and/or the nucleic acid residues 62 to 547 of SEQ ID No. 2, encoding amino acid residues 1 to 162 of SEQ ID No. 5 and 6. After cleavage of the signal peptide, this result in polypeptides containing amino acid residues 19 to 247 of SEQ ID No. 4 and amino acid residues 19 to 162 of SEQ ID No. 5 and 6.
In another embodiment, the nucleic acids encoding the anti-CD300LG-ext antibodies described above are directly administered in vivo, where they are expressed to produce the encoded products.

In vivo administration can be accomplished by any of numerous methods known in the art, e. g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e. g., by infection using defective or attenuated retroviral or other viral vectors (see U. S. Patent No. 4,980, 286), or by direct injection of naked DNA, or by use of microparticle bombardment (e. g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administrating it in linkage to a ligand subject to receptor-mediated endocytosis (see, e. g., Wu and Wu, 1987, J. 262: 4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e. g., PCT Publications WO 92/06180; WO 92/22635; W092/20316; W093/14188; WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA, 86: 8932-8935; and Zijistra 1989, Nature, 342: 435-438).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding CD300LG-ext polypeptides or anti-CD300LG-ext antibodies are used. For example, a retroviral vector can be used (see Miller et al., 1993, Methods Enzymol., 217:581-599).

These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA.

The nucleic acid sequences encoding the polypeptides of the invention to be used in gene therapy are cloned into one or more vectors, which facilitate delivery of the nucleotide sequence into a subject. Further details about retroviral vectors can be found in Boesen 1994, Biotherapy, 6: 291-302, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Cloves et al., 1994, J. Clin. Invest., 93:644-651; Klein et al., 1994, Blood, 83:1467-1473; Salmons and Gunzburg, 1993, Human Gene Therapy, 4: 129-141; and Grossman and Wilson, 1993, Curr. Opin. Genetics and devel., 3: 110-114.
Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle.


Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, gene transfer, fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, Meth. 217: 599-618; Cohen 1993, Enzymol., 217: 618-644; and Clin. Pharma. Ther., 29: 69-92, 1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.
Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include, but are not limited to, Dendritic cells, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e. g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding a polypeptide, an antibody or a fusion protein of the invention are introduced into the cells such that they are by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e. g., PCT Publication WO 94/08588; Stemple and Anderson, 1992, Cell, 71: 973-985; Rheinwald, 1980, Meth. Cell Bio., 21A: 229; and and Scott, 1986, Mayo Clinic Proc., 61: 771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

1. d. Nucleic acid agents inhibiting the expression of CD300LG-FL polypeptides.

For the induction of tolerance, the present invention also encompasses inhibition of CD300LG-FL polypeptide expression at a transcriptional and/or translational level. In particular, DNA sequences comprising nucleotides 96 to 782 of SEQ ID No. 1 and nucleotides 116 to 547 of SEQ ID No. 2 and 3 are the subject of modulation. In some embodiments the modulators are nucleic acid based, including, without limitations, DNA, RNA, chimeric RNA/DNA, protein nucleic acid (PNA), and other nucleic acid derivatives.

In one particular aspect, the CD300LG-FL polypeptide nucleic acid inhibitors encompass “small interfering RNAs” or “siRNAs”. These nucleic acids are about 19-30 nucleotides in length, such as e.g., about 21-23 nucleotides in length, corresponding in length to the fragments generated by nuclease “dicing” of longer double-stranded RNAs. The siRNAs are understood to recruit nuclease complexes and guide the complexes to the target mRNA by pairing to the specific sequences. As a result, the target mRNA is degraded by the nucleases in the protein complex. In a particular embodiment, the 21-23 nucleotides siRNA molecules comprise a 3’ hydroxyl group.
siRNA for use in the present invention can be obtained using a number of techniques known to those of skill in the art. For example, the siRNA can be chemically synthesized or recombinantly produced using methods known in the art. For example, short sense and antisense RNA oligomers can be synthesized and annealed to form double-stranded RNA structures with 2-nucleotide overhangs at each end (Caplen et al, Proc Natl Acad Sci USA, 98:9742-9747, 2001; and Elbashir et al, EMBOJ, 20:6877-88, 2001). These double-stranded siRNA structures can then be directly introduced to cells, either by passive uptake or by a delivery system of choice.

In certain embodiments, the siRNA constructs can be generated through the processing of longer double-stranded RNAs, for example, in the presence of the enzyme dicer. In one embodiment, the Drosophila in vitro system is used. In this embodiment, dsRNA is combined with a soluble extract derived from Drosophila embryo, thereby producing a combination. The combination is maintained under conditions in which the dsRNA is processed to RNA molecules of about 21 to about 23 nucleotides.

siRNA molecules can be purified using conventional techniques. For example, gel electrophoresis can be used to purify siRNAs. Alternatively, non-denaturing methods, such as non-denaturing column chromatography, can be used to purify siRNA. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, affinity purification with antibody can be used to purify siRNAs.

In yet another aspect, the CD300LG-FL polypeptide nucleic acid modulators of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup 1996, Bioorganic & Medicinal Chemistry, 4(1): 5-23). As used herein, the terms "peptide nucleic to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudo-peptide backbone and only the four are retained. The neutral backbone of PNA oligomers has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al., supra; Perry-O'Keefe et al., 1996, Proc. Natl. Acad. Sci. USA, 93: 14670-675.

PNAs can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigenic agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA
directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e. g., S1 nucleases (Hyrup, supra); or as probes or primers for DNA sequence and hybridization (Hyrup, supra; Perry-O'Keefe et al, supra).

In another embodiment, PNAs can be modified, e. g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated which can combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e. g., RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup, supra). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, supra, and Finn et al. (1996, Nucleic Acids Res., 24 (17): 3357-63). For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5'end of DNA (Mag et al., 1989, Nucleic Acids Res., 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5'PNA segment and a 3'DNA segment (Finn et al., 1996, Nucleic Acids Res., 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5'DNA segment and a 3'PNA segment (Petersen et al., 1975, Bioorganic Med. Chem. Lett., 5:1119-11124).

The present invention provides a pharmaceutical composition comprising polypeptides, nucleic acid molecules, and/or antibodies of the invention (herein also referred to as "active compounds"). The formulation may comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants.

In one aspect of the invention, a therapeutically effective amount of an active compound ranges from about 0.001 to 30 mg/kg body weight.

In one aspect of the invention, the dosage of the active compound is 0.1 to 100 mg/kg of body weight (generally 10 mg/kg mg/kg).

The present invention encompasses nucleic acid agents which inhibits expression, including but not limited to, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, having a molecular weight less than about 10,000 grams per molecule. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and
condition of the subject or being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained.

It is understood that the specific dose level of an active compound of the invention for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

In one embodiment of the invention, the pharmaceutical composition is an aqueous formulation, i.e., formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical composition is an aqueous solution. The term “aqueous formulation” is defined as a formulation comprising at least 50 %w/w water. Likewise, the term “aqueous solution” is defined as a solution comprising at least 50 %w/w water, and the term “aqueous suspension” is defined as a suspension comprising at least 50 %w/w water.

In one embodiment of the invention, the pharmaceutical composition is a freeze-dried formulation, where the physician or the patient adds solvents and/or diluents prior to use.

In one embodiment of the invention, the pharmaceutical composition is a dried formulation (e.g., freeze-dried or spray-dried) ready for use without any prior dissolution.

In one aspect, the invention relates to a pharmaceutical composition comprising an aqueous solution of active compounds, and a buffer, wherein said formulation has a pH from about 2.0 to about 10.0.
In one embodiment of the invention, the pH of the formulation is selected from the list consisting of 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, and 10.0.

In one embodiment of the invention, the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In one embodiment of the invention, the formulation further comprises a pharmaceutically acceptable preservative. In a further embodiment of the invention, the preservative is selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thimerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol) or mixtures thereof. In a further embodiment of the invention, the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In a further embodiment of the invention, the preservative is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention, the preservative is present in a concentration from 5 mg/ml to 10 mg/ml. In a further embodiment of the invention, the preservative is present in a concentration from 10 mg/ml to 20 mg/ml. Each one of these specific preservatives constitutes an alternative embodiment of the invention. The use of a preservative in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

In one embodiment of the invention, the formulation further comprises an isotonic agent. In a further embodiment of the invention, the isotonic agent is selected from the group consisting of a salt (e.g. sodium chloride), a sugar or sugar alcohol, an amino acid (e.g. L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (e.g. glycerol (glycerine), 1,2-propanediol (propylene glycol), 1,3-propanediol, 1,3-butanediol) polyethyleneglycol (e.g. PEG400), or mixtures thereof. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose,
mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na may be used. In one embodiment the sugar additive is sucrose. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one -OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment the sugar alcohol additive is mannitol. The sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid preparation and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the sugar or sugar alcohol concentration is between about 1 mg/ml and about 150 mg/ml. In a further embodiment of the invention, the isotonic agent is present in a concentration from 1 mg/ml to 50 mg/ml. In a further embodiment of the invention, the isotonic agent is present in a concentration from 1 mg/ml to 7 mg/ml. In a further embodiment of the invention, the isotonic agent is present in a concentration from 8 mg/ml to 24 mg/ml. In a further embodiment of the invention, the isotonic agent is present in a concentration from 25 mg/ml to 50 mg/ml. Each one of these specific isotonic agents constitutes an alternative embodiment of the invention. The use of an isotonic agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In one embodiment of the invention, the formulation further comprises a chelating agent. In a further embodiment of the invention, the chelating agent is selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. In a further embodiment of the invention, the chelating agent is present in a concentration from 0.1 mg/ml to 5 mg/ml. In a further embodiment of the invention, the chelating agent is present in a concentration from 0.1 mg/ml to 2 mg/ml. In a further embodiment of the invention, the chelating agent is present in a concentration from 2 mg/ml to 5 mg/ml. Each one of these specific chelating agents constitutes an alternative embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In one embodiment of the invention, the formulation further comprises a stabilizer. The use of a stabilizer in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.
Pharmaceutical compositions of the invention may be stabilized liquid pharmaceutical compositions whose therapeutically active components include a polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical compositions. By "aggregate formation" is intended a physical interaction between the polypeptide molecules that results in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. By "during storage" is intended a liquid pharmaceutical composition or formulation once prepared, is not immediately administered to a subject. Rather, following preparation, it is packaged for storage, either in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is intended the liquid pharmaceutical composition or formulation is dried either by freeze drying (i.e., lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38:48-59), spray drying (see Masters (1991) in Spray-Drying Handbook (5th ed; Longman Scientific and Technical, Essex, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18:1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11:12-20), or air drying (Carpenter and Crowe (1988) Cryobiology 25:459-470; and Roser (1991) Biopharm. 4:47-53). Aggregate formation by a polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

The pharmaceutical compositions of the invention may further comprise an amount of an amino acid base sufficient to decrease aggregate formation by the polypeptide during storage of the composition. By "amino acid base" is intended an amino acid or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids to use in preparing the compositions of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid, and glutamic acid. Any stereoisomer (i.e., L, D, or a mixture thereof) of a particular amino acid (e.g. methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment the L-stereoisomer is used. Compositions of the invention may also be
formulated with analogues of these amino acids. By "amino acid analogue" is intended a derivative of the naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the polypeptide during storage of the liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl L-arginine, suitable methionine analogues include ethionine and buthionine and suitable cysteine analogues include S-methyl-L cysteine. As with the other amino acids, the amino acid analogues are incorporated into the compositions in either their free base form or their salt form. In a further embodiment of the invention, the amino acids or amino acid analogues are used in a concentration, which is sufficient to prevent or delay aggregation of the protein.

In one embodiment of the invention, methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. By "inhibit" is intended minimal accumulation of methionine oxidized species over time. Inhibiting methionine oxidation results in greater retention of the polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of the methionine residues such that the amount of methionine sulfoxide is acceptable to regulatory agencies. Typically, this means that the composition contains no more than about 10% to about 30% methionine sulfoxide. Generally, this can be achieved by adding methionine such that the ratio of methionine added to methionine residues ranges from about 1:1 to about 1000:1, such as 10:1 to about 100:1.

In one embodiment of the invention, the formulation further comprises a stabilizer selected from the group of high molecular weight polymers or low molecular compounds. In a further embodiment of the invention, the stabilizer is selected from polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivates thereof (e.g. HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (e.g. sodium chloride). Each one of these specific stabilizers constitutes an alternative embodiment of the invention.

The pharmaceutical compositions may also comprise additional stabilizing agents, which further enhance stability of a therapeutically active polypeptide therein. Stabilizing agents of particular interest to the present invention include, but are not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a
nonionic surfactant, which protects the polypeptide against aggregation associated with freeze-thawing or mechanical shearing.

In one embodiment of the invention, the formulation further comprises a surfactant. In a further embodiment of the invention, the surfactant is selected from a detergent, ethoxylated castor oil, polyglycolyzed glycerides, acetylated monoglycerides, sorbitan fatty acid esters, polyoxypropylene-polyoxyethylene block polymers (e.g. poloxamers such as Pluronic® F68, poloxamer 188 and 407, Triton X-100), polyoxyethylene sorbitan fatty acid esters, polyoxyethylene and polyethylene derivatives such as alkylated and alkoxylated derivatives (twents, e.g. Tween-20, Tween-40, Tween-80 and Brij-35), monoglycerides or ethoxylated derivatives thereof, diglycerides or polyoxyethylene derivatives thereof, alcohols, glycerol, lecithins and phospholipids (e.g. phosphatidyl serine, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, diphostatidyl glycerol and sphingomyelin), derivatives of phospholipids (e.g. dipalmitoyl phosphatidic acid) and lysophospholipids (e.g. palmitoyl lysophosphatidyl-L-serine and 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine) and alkyl, alkoxy (alkyl ester), alkoxy (alkyl ether)- derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, and the positively charged DODAC, DOTMA, DCP, BISHOP, lysophosphatidylserine and lysophosphatidylthreonine, and glycerophospholipids (e.g. cephalins), glyceroglycolipids (e.g. galactopyranoside), sphingoglycolipids (e.g. ceramides, gangliosides), dodecylphosphocholine, hen egg lysolecithin, fusidic acid derivatives- (e.g. sodium tauro-dihydrofusidate etc.), long-chain fatty acids and salts thereof C6-C12 (e.g. oleic acid and caprylic acid), acylcarnitines and derivatives, N°-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N°-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N°-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulphate or sodium lauryl sulphate), sodium caprylate, cholic acid or derivatives thereof, bile acids and salts thereof and glycine or taurine conjugates, ursodeoxycholic acid, sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, zwitterionic surfactants (e.g. N-alkyl-N,N-dimethylammonio-1-propanesulfonates,
3-cholamido-1-propyldimethylammonio-1-propanesulfonate, cationic surfactants (quaternary ammonium bases) (e.g. cetyltrimethylammonium bromide, cetylpyridinium chloride), non-ionic surfactants (e.g. Dodecyl β-D-glucopyranoside), poloxamines (e.g. Tetronic's), which are tetrafunctional block copolymers derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, or the surfactant may be selected from the group of imidazoline derivatives, or mixtures thereof. Each one of these specific surfactants constitutes an alternative embodiment of the invention.

The use of a surfactant in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In one embodiment of the invention, the formulation further comprises protease inhibitors such as EDTA (ethylenediamine tetraacetic acid) and benzamidineHCl, but other commercially available protease inhibitors may also be used. The use of a protease inhibitor is particular useful in pharmaceutical compositions comprising zymogens of proteases in order to inhibit autocatalysis.

It is possible that other ingredients may be present in a pharmaceutical composition of the present invention. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical composition of the present invention.

Pharmaceutical compositions containing an active agent according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen.

Administration of pharmaceutical compositions according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.
Pharmaceutical compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

Pharmaceutical compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the active agent of the invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticles, liquid crystals and dispersions thereof, L2 phase and dispersions thereof, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Pharmaceutical compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of the active agent, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all being devices well known to those skilled in the art.

Pharmaceutical compositions of the current invention are specifically useful in the formulation of controlled, sustained, protracting, retarded, and slow release drug delivery systems. More specifically, but not limited to, compositions are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneous. Without limiting the scope of the invention, examples of useful controlled
release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles,

Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension for the administration of the active compounds in the form of a nasal or pulmonal spray. As a still further option, the pharmaceutical compositions containing the active agent of the invention can also be adapted to transdermal administration, e.g. by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, e.g. buccal, administration.

The active compounds can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise of, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebulizers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien YW.


Based on standardised testing methodology, the aerodynamic diameter (\(d_a\)) of a particle is defined as the geometric equivalent diameter of a reference standard spherical particle of unit density (1 g/cm\(^3\)). In the simplest case, for spherical particles, \(d_a\) is related to a reference diameter (\(d\)) as a function of the square root of the density ratio as described by:

\[
d_a = \sqrt{\frac{d}{\rho / \rho_a}}
\]

are well-described and known to the art (cf. Edwards DA, Ben-Jebrina A, Langer R and represents a measure of the median value of an aerodynamic particle size distribution. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). Mass median aerodynamic diameter (MMAD) and mass median effective aerodynamic diameter (MMEAD) are used interchangeably, are statistical parameters, and empirically describe the size of aerosol particles in relation to their potential to deposit in the lungs, independent of actual shape, size, or density (cf. Edwards DA, Ben-Jebra A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). MMAD is normally calculated from the measurement made with impactors, an instrument that measures the particle inertial behaviour in air.

In one embodiment, the formulation could be aerosolized by any known aerosolization technology, such as nebulisation, to achieve a MMAD of aerosol particles less than 10 μm, more preferably between 1-5 μm, and most preferably between 1-3 μm. The preferred particle size is based on the most effective size for delivery of drug to the deep lung, where protein is optimally absorbed (cf. Edwards DA, Ben-Jebra A, Langer A. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385).

Deep lung deposition of the pulmonary formulations comprising the active compounds may be further optimized by using modifications of the inhalation techniques, for example, but not limited to: slow inhalation flow (e.g. 30 L/min), breath holding and timing of actuation.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability.

The term "physical stability" of the protein formulation as used herein refers to the tendency of the protein to form biologically inactive and/or insoluble aggregates of the protein as a result of exposure of the protein to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of the aqueous protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation filled in suitable containers (e.g. cartridges or vials) to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Visual inspection of the formulations is performed in a sharp focused light with a dark background. The turbidity of the formulation is characterized by a visual score ranking the degree of turbidity for instance on a scale from 0 to 3 (a formulation showing no turbidity corresponds to a visual score 0, and a formulation showing
visual turbidity in daylight corresponds to visual score 3). A formulation is classified physical unstable with respect to protein aggregation, when it shows visual turbidity in daylight. Alternatively, the turbidity of the formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of the aqueous protein formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the protein. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the protein. One example of a small molecular spectroscopic probe of protein structure is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and perhaps other protein configurations as well, Thioflavin T gives rise to a new excitation maximum at about 450 nm and enhanced emission at about 482 nm when bound to a fibril protein form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths.

Other small molecules can be used as probes of the changes in protein structure from native to non-native states. For instance the “hydrophobic patch” probes that bind preferentially to exposed hydrophobic patches of a protein. The hydrophobic patches are generally buried within the tertiary structure of a protein in its native state, but become exposed as a protein begins to unfold or denature. Examples of these small molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as anthracene, acridine, phenanthroline or the like. Other spectroscopic probes are metal-amino acid complexes, such as cobalt metal complexes of hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, methionine, and valine, or the like.

The term “chemical stability” of the protein formulation as used herein refers to chemical covalent changes in the protein structure leading to formation of chemical degradation products with potential less biological potency and/or potential increased immunogenic properties compared to the native protein structure. Various chemical degradation products can be formed depending on the type and nature of the native protein and the environment to which the protein is exposed. Elimination of chemical degradation can most probably not be completely avoided and increasing amounts of chemical degradation products is often seen during storage and use of the protein formulation as well-known by the person skilled in the art. Most proteins are prone to deamidation, a process in which the side chain amide group in glutaminyl or asparaginyl residues is hydrolysed to form a free carboxylic acid. Other degradations pathways involves formation of high molecular weight transformation products where two or more protein molecules are covalently bound to each other through transamidation and/or disulfide interactions leading to formation of
covalently bound dimer, oligomer and polymer degradation products (Stability of Protein Pharmaceuticals, Ahem. T.J. & Manning M.C., Plenum Press, New York 1992). Oxidation (of for instance methionine residues) can be mentioned as another variant of chemical degradation. The chemical stability of the protein formulation can be evaluated by measuring the amount of the chemical degradation products at various time-points after exposure to different environmental conditions (the formation of degradation products can often be accelerated by for instance increasing temperature). The amount of each individual degradation product is often determined by separation of the degradation products depending on molecule size and/or charge using various chromatography techniques (e.g. SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

In one embodiment of the invention, the pharmaceutical composition comprising the active compounds are stable for more than 6 weeks of usage and for more than 3 years of storage.

In one embodiment of the invention, the pharmaceutical composition comprising the active compounds are stable for more than 4 weeks of usage and for more than 3 years of storage.

In one embodiment of the invention, the pharmaceutical composition comprising the active compound are stable for more than 4 weeks of usage and for more than two years of storage.

In one embodiment of the invention, the pharmaceutical composition comprising the active compounds are stable for more than 2 weeks of usage and for more than two years of storage.

The proteins, antibodies, and nucleic acid molecules described herein can be used in one or more of the following methods: a) predictive medicine (e.g., diagnostic assays and prognostic assays) and b) methods of treatment (e.g., therapeutic and prophylactic). For example, polypeptides of the invention can be used to (i) modulate cellular proliferation and/or (iii) modulate cellular adhesion. The isolated nucleic acid molecules of the invention can be used to express proteins (e.g., via a recombinant expression vector in a host cell and/or in gene therapy applications). Alternatively, modified isolated nucleic acids of the invention, such as siRNAs, can be used to down-regulate the expression of CD300LG-FL polypeptide. The polypeptides of the invention can be used to treat disorders characterized
by insufficient or excessive production of CD300LG-FL polypeptides or production of a form of a protein of the invention which has decreased or aberrant activity compared to the wild type protein. Finally, the antibodies of the invention can be used to detect and isolate a protein of the invention as well as CD300LG-FL polypeptides and/or modulate the activity of a protein of the invention as well as CD300LG-FL polypeptides.

In one aspect, the invention relates to an isolated CD300LG-ext polypeptide according to the invention for use in medicine.

In another aspect, the invention relates to a pharmaceutical composition comprising an isolated CD300LG-ext polypeptide according to the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention relates to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the induction of tolerogenic dendritic cells in a subject.

In another aspect, the invention relates to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival.

In another aspect, the invention relates to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of autoimmune diseases.

In another aspect, the invention relates to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of allergic hypersensitivity.

In another aspect, the invention relates to the use of an isolated CD300LG-ext polypeptide according to the invention for the production of a medicament for the prolongation of allograft survival in a subject in need thereof.

In one aspect of the invention, the use of a CD300LG-ext polypeptide according to the invention for the preparation of a medicament for treating a subject having a disease or disorder associated with increased full-length CD300LG expression, is provided.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide
according to the invention for the production of a medicament for the induction of tolerogenic DC’s.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of one or more diseases or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and myasthenia gravis.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of autoimmune diseases.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament for the treatment or prevention of allergic hypersensitivity.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament for prolongation of allograft survival in a subject in need of such.

In another aspect, the invention relates to the use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide according to the invention for the production of a medicament wherein the medicament comprising the DNA encoding the polypeptide is expressing the polypeptide \textit{in vivo}.

In another aspect, the invention relates to the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for reduced transcription or translation and thereby reduced expression of CD300LG-FL protein \textit{in vivo} after administration.

In another aspect, the invention relates to the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for the treatment or prevention of one or more diseases or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as
Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and myasthenia gravis.

In another aspect, the invention relates to the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for treatment of an autoimmune disease.

In another aspect, the invention relates to the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for treatment or prevention of allergic hypersensitivity.

In another aspect, the invention relates to the use of DNA encoding CD300LG-ext polypeptide according to the invention for the generation of an agent, including siRNA, for the preparation of a medicament for prolongation of allograft survival in a subject in need of such.

In one aspect, the invention relates to a pharmaceutical composition comprising an inhibitory antibody or a fragment thereof according to the invention and a pharmaceutically acceptable carrier.

In another aspect of the invention, the use of an inhibitory antibody or a fragment thereof according to the invention for the production of a medicament for the induction of tolerance of dendritic cells, is provided.

In another aspect of the invention, the use of an inhibitory antibody or a fragment thereof according to the invention for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival, is provided.

In another aspect, the invention relates to the use an inhibitory antibody or a fragment thereof according to the invention for the production of a medicament for the treatment or prevention of autoimmune diseases.

In another aspect, the invention relates to the use of an inhibitory antibody or a fragment thereof according to the invention for the production of a medicament for the treatment or prevention of allergic hypersensitivity.
In another aspect, the invention relates to the use of an inhibitory antibody or a fragment thereof according to the invention for the production of a medicament for the prolongation of allograft survival in a subject in need thereof.

In one aspect of the invention, the use of an inhibitory antibody or a fragment thereof according to the invention for the preparation of a medicament for treating a subject having a disease or disorder associated with increased full-length CD300LG expression, is provided.

In another aspect, the invention relates to a pharmaceutical composition comprising an activating antibody or a fragment thereof according to the invention and a pharmaceutically acceptable carrier.

In another aspect of the invention, the use of an activating antibody or a fragment thereof according to the invention for the production of a medicament for the reduction of tolerance of dendritic cells, is provided.

In another aspect of the invention, the use of an activating antibody or a fragment thereof according to the invention for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting of cancer or infections, is provided.

In another aspect of the invention, the use of an activating antibody or a fragment thereof according to the invention for the preparation of a medicament for treating a subject having a disease or disorder associated with reduced CD300LG full-length expression, is provided.

A more individualized diagnosis is needed in a variety of autoimmune diseases. The presently available diagnostic tools will in many cases fail to predict whether or not a patient will benefit from a given treatment and strategies to select patients who are likely to benefit from specific therapies are needed. A biomarker to assess the "tolerogenic" state of a patient would be very useful. Also a more individualized diagnosis is needed in a variety of malignancies. Particularly in cancer this is evident, where markedly different treatment responses and overall outcomes are observed in patients categorized presently to be in apparently similar diseased states. Gene expression profiles have successfully been used for prediction of clinical outcome of breast cancer (Van’t Veer et al., 2002) and central nervous system embryonal tumors (Pomeroy et al., 2002).

One aspect of the present invention relates to diagnostic assays for determining expression of CD300LG-FL- and/or CD300LG-ext-polypeptides, in the context of a biological sample (e.g., blood, plasma, serum, cells, tissues) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant expression or activity of CD300LG-FL polypeptides, such as an
autoimmune disorder, allergic hypersensitivity, infections, or cancer. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with aberrant expression or activity of CD300LG-FL polypeptides. The invention can be carried out by measuring the protein level of CD300LG-FL in dendritic cells from patients suffering from the autoimmune disease of relevance, from infections, or from cancer and correlating the level of CD300LG-FL polypeptides to the progression of disease. A correlation between low levels of CD300LG-FL and slow progression of disease in autoimmune patients would indicate that CD300LG-FL may be used as a biomarker in autoimmune diseases. Similarly, a correlation between high levels of CD300LG-FL and slow progression of disease in cancer patients would indicate that CD300LG-FL may be used as a biomarker in cancer.

A preferred agent for detecting a polypeptide of the invention is an antibody capable of binding to a polypeptide of the invention, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g. Fab or F (ab') 2) can be used. See also the detailed descriptions about antibodies above. For example, in vitro techniques for detection of a polypeptide of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations. Furthermore, in vivo techniques for detection of a polypeptide of the invention include introducing into a subject a labeled antibody directed against the polypeptide. For example, the antibody can be labelled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

An important function of DCs is the ability to stimulate the proliferation of T cells, reflecting the tolerogenic and stimulatory state of the DCs. The state of DCs can be analysed by a so-called mixed leucocyte reaction (MLR), which is an in vitro model for T cell activation. Besides T cell proliferation, a typical feature of these MLR-assays is the formation of large DC-T cell-clusters. Effects of CD300LG-ext polypeptides of the invention, antibodies raised against the polypeptides of the invention as well as DNA agents such as siRNAs, inhibiting CD300LG-FL function (collectively referred to as active compounds) can be studied by the use of MLR assays. In practicing the MLR assays, the active compounds may be administered such that they produce a measurable statistical change in cpm as well as numbers of DC-T cell clusters.

The present invention provides methods for preventing and/or treating inflammatory diseases, including various inflammatory autoimmune disorders, allergic hypersensitivity, allograft rejection and/or syndromes associated with increased CD300LG-FL polypeptide function on DCs. In one aspect of the invention, an inhibitor of CD300LG-FL polypeptide
function may be administered to a patient such that it produces a measurable statistical improvement in outcome, as evidenced by at least one clinical parameter associated with the syndrome.

Accordingly, the invention provides in one aspect a method for treating and/or preventing rheumatoid arthritis (RA). The method comprises delivering an effective amount of an agent that reduces CD300LG-FL polypeptide function to a patient having RA or being identified/diagnosed as being at substantial risk of developing RA, such that RA is treated or prevented. In one aspect, the inventive RA treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibit the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. In one aspect of the invention the active compound are demonstrated to be effective in ameliorating RA in an acceptable model of RA, such as is described in US Patent No. 6,414,218, US Patent Publication No. 20030005469, and Wooley, P.H. animal models of arthritis, eds. J.H. Klippel and P.A. Dieppe, Mosby Publishers (London), 1998. The effect of the treatment can be measured as a reduction in disease signs including a reduced swelling of affected joints, by a histological investigation of the affected joints, mobility, and/or the official disease score ACR 20/50 or 70.

In another aspect, the invention provides a method for treating and/or preventing multiple sclerosis (MS). The method comprises delivering an effective amount of an agent that reduces CD300LG-FL function to a patient having MS or being identified/diagnosed as being at substantial risk of developing MS, such that MS is treated or prevented. In one aspect, the inventive MS treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational
and/or post-translational level. In one aspect of the invention the active compound are demonstrated to be effective in ameliorating MS in an acceptable model of MS, such as Chronic progressive or relapsing-remitting models of experimental autoimmune encephalomyelitis induced by immunization with immunodominant peptides derived from myeloidrodendrocyte (MOG) protein, proteolipid protein (PLP) or myelin basic protein (MBP) administered in complete Freund’s adjuvant (CFA) with or without pertussis toxin. The effect of the treatment can be measured as a reduction in disease signs including weight loss, paralysis of the tail and limbs, brain inflammation assessed through scanning of the brain, and by a histological investigation of the CNS and spine.

In another aspect, the invention provides a method for treating and/or preventing inflammatory bowel disease (IBD), such as Chron’s disease or ulcerative colitis. The method comprises delivering an effective amount of an agent that reduces CD300LG-FL function to a patient having IBD or being identified/diagnosed as being at substantial risk of developing IBD, such that IBD is treated or prevented. In one aspect, the inventive IBD treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. In one aspect of the invention the active compound are demonstrated to be effective in ameliorating IBD in an acceptable model of IBD, such as TNBS-induced colitis, oxazolone-induced colitis, DSS-induced colitis, IL-10 deficient mice, immune deficient mice (RAG-deficient and SCID mice) adoptively transferred with syngeneic CD4+CD25- or CD4+CD45RBhigh T cells and SAMP/Yit mice. The effect of the treatment can be measured as a reduction in disease signs including delayed and/or less severe diarrhoea, delayed alterations in the weight/length ratio for the colon, delayed weight loss and by a histological investigation of the affected parts of the gut.

In another aspect, the invention provides a method for treating and/or preventing insulin-dependent diabetes mellitus (IDDM). The method comprises delivering an effective amount of an agent that reduces CD300LG-FL function to a patient suffering from or at substantial risk of developing IDDM, in an amount and under conditions sufficient to treat or prevent the condition in the patient or host. In one aspect, the inventive IDDM
treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. In one aspect of the invention the active compound are demonstrated to be effective in ameliorating IDDM in an acceptable model of IDDM, such as BB rat and BB-rat derived models, nonobese diabetic (NOD) mice, NOD-SCID mice adoptively transferred with spleen cells or purified T cells from diabetic NOD mice, RIP-LCMV transgenic mice and multiple low dose streptozotocin-induced diabetic mice. The effect of the treatment can be evaluated by measuring blood glucose levels or variations thereof, Hb1C levels, and the like and can be measured as a reduction in disease signs including delayed onset of hyperglycemia, diminished incidence of hyperglycemia or restoration of normoglycemia, and by histological investigation of the islets of Langerhans.

In another aspect, the invention provides a method for treating and/or preventing systemic lupus erythematosus (SLE). The method comprises delivering an effective amount of an agent that reduces CD300LG-FL function to a patient suffering from or at substantial risk of developing SLE, in an amount and under conditions sufficient to treat or prevent the condition in the patient or host. In one aspect, the inventive SLE treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. In one aspect of the invention the active compound are demonstrated to be effective in ameliorating SLE in an acceptable model of SLE, such as (NZW x NZB)F1 mice, MRL-Fas<sup>lor</sup> mice and Bxsb-YAA. The effect of the treatment can be measured as diminished serum auto-antibodies against dsDNA, diminished serum IgG, diminished skin lesion, diminished
lymphadenopathy, diminished proteinuria, diminished glumerular lg deposits and diminished thickening of the glomerular basement membrane.

In yet another aspect, the invention provides a method for treating and/or preventing psoriasis. In one aspect, the inventive psoriasis treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In one aspect, the inventive psoriasis treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that "specifically binds to" CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. The active agents are administered prophylactically or therapeutically to immune deficient engrafted with skin biopsies from both non-affected as well as diseased areas from psoriatic patients. The effect of the treatment can be measured histologically as diminished recruitment of leukocytes to the graft, diminished reddening and diminished thickening of the graft.

In another facet, the invention provides a method for treating and/or preventing allergic hypersensitivity. In one aspect, the inventive allergic hypersensitivity treatment/prevention method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that "specifically binds to" CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. The active agents are can be tested in either of the following models: 1) Mice immunized subcutaneously with ovalbumin in CFA and re-challenged by intra-bronchial instillation of ovalbumin as model for asthma. The effect of the treatment can be measured as an increased pulmonary capacity, as a reduction of inflammatory cytokines and leukocytes in the lungs and bronchoalveolar lavage and by histological investigation of the
lungs. 2) Delayed-type hypersensitivity (DTH) induced by immunization with a hapten (TNBS, oxazolone, FITC, TNB) followed by painting of the skin of the footpad or ear with the same hapten. The effect of the treatment can be measured as a reduced swelling of the ears or footpad and histologically as reduced recruitment of leukocytes to the affected tissue. 3) Contact hypersensitivity (CHS) induced by painting of the skin with the hapten (TNFB, oxazolone, FITC, TNB) and rechallenge by painting of the skin of the ear with the same hapten as model for allergic hypersensitivity. The effect of the treatment can be measured as a reduced swelling of the ears and histologically as reduced recruitment of leukocytes to the affected tissue.

In yet another facet, the invention provides methods of reducing the likelihood of transplant rejections and prolongation of allograft survival. In some preferred embodiments, the transplant is a bone marrow (BM) or peripheral blood stem cell (PBSM) transplant. In some embodiments, the BMT or PBSCT transplant is administered as treatment of leukemia or lymphoma, while in other embodiments, the transplant is administered as treatment for other types of cancers such as neuroblastoma or multiple myeloma. In one aspect, the inventive transplant survival method is practiced by use of soluble forms of CD300LG polypeptides (CD300LG-ext polypeptides) that specifically inhibits the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In a further aspect, the agent is an antibody that “specifically binds to” CD300LG-ext polypeptides and are capable of inhibiting the interaction between CD300LG-FL polypeptides and their corresponding ligands/receptors. In an additional aspect, the agent is a modulator (e.g. siRNA) preventing CD300LG-FL polypeptides from reaching the cell surface, including interfering with the production of CD300LG-FL polypeptides at a transcriptional, translational and/or post-translational level. The active agents can be tested in mouse models of allograft survival by administering prophylactically or therapeutically the agents to mice engrafted with allogeneic heart, skin or islets of Langerhans. The effect of the treatment can be measured as prolonged survival of the graft. For hematopoietic transplant rejections, severity of the disease (failure to engraft) and/or outcome of treatment may be evaluated by evidence of prolonged neutropenia, thrombocytopenia, and red-cell transfusion dependence in patients that have undergone myeloablative conditions.

The inventive method can similarly be applied to a variety of other autoimmune diseases and inflammatory conditions associated with CD300LG, including Hashimoto’s thyroiditis, myasthenia gravis, Guillain-Barré syndrome, autoimmune uveitis, primary biliary cirrhosis, autoimmune hepatitis, autoimmune hemolytic anemia, pernicious anemia, autoimmune thrombocytopenia, Grave’s disease, autoimmune oophoritis, autoimmune
orchitis, temporal arteritis, anti-phospholipid syndrome, Wegener's granulomatosis, Behcet's disease, scleroderma, polymyositis, dermatomyositis, ankylosing spondylitis, Sjögren's syndrome, dermatitis herpetiformis, pemphigus vulgaris, vitiligo, psoriatic arthritis, osteoarthritis, steroid-resistant asthma, chronic obstructive pulmonary disease and atherosclerosis.

The present invention provides methods for preventing and/or treating infections and cancer associated with decreased CD300LG-FL polypeptide function on DCs and thereby increased tolerance. In practicing the present invention, an activator of CD300LG-FL polypeptide function may be administered to a patient such that it produces a measurable statistical improvement in outcome, as evidenced by at least one clinical parameter associated with the syndrome.

Accordingly, the invention provides a method for treating and/or preventing infections. The method comprises delivering an effective amount of an agent that increases CD300LG-FL polypeptide function to a patient suffering from or at substantial risk of developing infections, in an amount and under conditions sufficient to treat or prevent the condition in the patient or host. In one aspect, the inventive infection treatment/prevention method is practiced by use of an antibody that "specifically binds to" CD300LG-ext polypeptides and are capable of activating CD300LG-FL polypeptides. In another aspect, the inventive infection treatment/prevention method is practiced by use of an antibody that "specifically binds to" the ligand/receptor for CD300LG polypeptides and capable of activating CD300LG ligand/receptor polypeptides. The antibodies are demonstrated to be effective in ameliorating infections, and the invention can be carried out in mouse models by administering prophylactically or therapeutically the antibody to mice infected with relevant pathogens. The effect of the treatment can be measured as reduced virulence, reduced lethality or prolonged survival time of the mice.

In yet another aspect, the invention provides a method for treating and/or preventing cancer. The method comprises delivering an effective amount of an agent that increases CD300LG-FL polypeptide function to a patient suffering from or at substantial risk of developing cancer, in an amount and under conditions sufficient to treat or prevent the condition in the patient or host. In one aspect, the inventive infection treatment/prevention method is practiced by use of an antibody that "specifically binds to" CD300LG-ext polypeptides and are capable of activating CD300LG-FL polypeptides. In another aspect, the inventive infection treatment/prevention method is practiced by use of an antibody that "specifically binds to" the ligand/receptor for CD300LG polypeptides and capable of activating CD300LG ligand/receptor polypeptides. The antibodies are demonstrated to be
effective in ameliorating infections, and the invention can be carried out in mouse models of
allograft survival by administering prophylactically or therapeutically the antibody to mice into
which tumors are induced, either by treatment with carcinogenic compounds or by
engraftment of syngeneic or xenogeneic tumors. The effect of the treatment can be
measured as reduced growth rate of the tumors, reduced number of tumor nodules or
metastasis or prolonged survival time.

The following is a list of embodiments of the present invention:

Embodiment 1. An isolated CD300LG-ext polypeptide having the amino acid
sequence of amino acids 19-247 of SEQ ID No. 4, a variant, a splice variant, a fragment or a
derivative thereof.

Embodiment 2. A CD300LG-ext polypeptide according to embodiment 1 having the
amino acid sequence of amino acids 19-247 of SEQ ID No. 4.

Embodiment 3. A CD300LG-ext polypeptide according to embodiment 1, which is a
variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-
247 of SEQ ID No. 4.

Embodiment 4. A CD300LG-ext polypeptide according to embodiment 1 having an
amino acid sequence, which sequence has at least 90% identity to the amino acid sequence
of amino acids 19-247 of SEQ ID No. 4.

Embodiment 5. A CD300LG-ext polypeptide according to embodiment 4 having an
amino acid sequence, which sequence has at least 95% identity to the amino acid sequence
of amino acids 19-247 of SEQ ID No. 4.

Embodiment 6. A CD300LG-ext polypeptide according to embodiment 5 having an
amino acid sequence, which sequence has at least 98% identity to the amino acid sequence
of amino acids 19-247 of SEQ ID No. 4.

Embodiment 7. A CD300LG-ext polypeptide according to embodiment 1, which is a
splice variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids
19-247 of SEQ ID No. 4.

Embodiment 8. A CD300LG-ext polypeptide according to embodiment 1, which is a
fragment of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-
247 of SEQ ID No. 4.

Embodiment 9. A CD300LG-ext polypeptide according to embodiment 1, which is a
derivative of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-
247 of SEQ ID No. 4.
Embodiment 10. An isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6, a variant, a splice variant, a fragment or derivative thereof.

Embodiment 11. A CD300LG-ext polypeptide according to embodiment 10 having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 12. A CD300LG-ext polypeptide according to embodiment 10, which is a variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 13. A CD300LG-ext polypeptide according to embodiment 10 having an amino acid sequence, which sequence has at least 90% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 14. A CD300LG-ext polypeptide according to embodiment 13 having an amino acid sequence, which sequence has at least 95% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 15. A CD300LG-ext polypeptide according to embodiment 14 having an amino acid sequence, which sequence has at least 98% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 16. A CD300LG-ext polypeptide according to embodiment 10, which is a splice variant of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 17. A CD300LG-ext polypeptide according to embodiment 10 which is a fragment of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 18. A CD300LG-ext polypeptide according to embodiment 10, which is a derivative of a CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 19. A CD300LG-ext polypeptide according to any of embodiments 1 to 9 which further comprises one or more selected from the group consisting of:

(i) one or more amino acid residues derived from the signal sequence of amino acids 1-18 of SEQ ID No. 4 at its N-terminus,

(ii) one or more amino acid residues derived from the transmembrane domain at its C-terminus, preferably amino acid residues 248-251 (ILAP) of SEQ ID No. 4,

(iii) functional sequences attached to its N-terminus, or

(iv) functional sequences attached to its C-terminus.
Embodiment 20. A CD300LG-ext polypeptide according to embodiment 1 or any of embodiments 10 to 18, which further comprises one or more selected from the group consisting of:

(i) one or more amino acid residues derived from the signal sequence of amino acids 1-18 of SEQ ID No. 5 or 6 at its N-terminus,

(ii) one or more amino acid residues derived from the neighboring transmembrane domain at its C-terminus, preferably amino acid residues 163-166 (ILAP) of sequence ID No. 5 or 6,

(iii) functional sequences attached to its N-terminus, or

(iv) functional sequences attached to its C-terminus.

Embodiment 21. A CD300LG-ext polypeptide according to embodiment 19, wherein the CD300LG-ext polypeptide further has an amino acid residue 18 at the N-terminus of amino acids 19-247 of SEQ ID No. 4.

Embodiment 22. A CD300LG-ext polypeptide according to embodiment 21, wherein the amino acid residue at the N-terminus of amino acids 19-247 of SEQ ID No. 4 is an alanine.

Embodiment 23. The CD300LG-ext polypeptide according to embodiment 20, wherein the CD300LG-ext polypeptide further has an amino acid residue 18 at the N-terminus of amino acids 19-162 of SEQ ID No. 5 or 6.

Embodiment 24. A CD300LG-ext polypeptide according to embodiment 23, wherein the amino acid residue at the N-terminus of amino acids 19-162 of SEQ ID No. 5 or 6 is an alanine.

Embodiment 25. A CD300LG-ext polypeptide according to any of embodiments 1 to 24, which inhibits DC-dependent allogenic T cell proliferation as described in assays of example 6 and 7 and/or inhibits DC-T cell clustering as described in assay of example 8.

Embodiment 26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide of any of embodiments 1 to 25.

Embodiment 27. A nucleic acid molecule according to embodiment 26, wherein the molecule is DNA.

Embodiment 28. A nucleic acid molecule according to embodiment 26, wherein the molecule is RNA.

Embodiment 29. A nucleic acid molecule according to embodiment 27 having a nucleotide sequence of 42-782 of SEQ ID No. 1.

Embodiment 30. A nucleic acid molecule according to embodiment 27 having a nucleotide sequence of 96-782 of SEQ ID No. 1.
Embodiment 31. A nucleic acid molecule according to embodiment 27 having a nucleotide sequence of 62-547 of SEQ ID No. 2.

Embodiment 32. A nucleic acid molecule according to embodiment 27 having a nucleotide sequence of 116-547 SEQ ID No. 2.

Embodiment 33. A vector containing a nucleic acid molecule of any of embodiments 27 to 32.

Embodiment 34. A host cell comprising the vector of embodiment 33.

Embodiment 35. A host cell comprising a nucleic acid molecule of embodiment 27 or any of embodiments 29-32 operably linked to a heterologous promoter.

Embodiment 36. A host cell of embodiment 35 which is a prokaryotic cell.

Embodiment 37. A host cell of embodiment 35 which is a eukaryotic cell.

Embodiment 38. A method for producing a polypeptide as defined in any of embodiments 1 to 25 comprising expressing the polypeptide encoded by the nucleic acid molecule of the host cell of embodiment 34 to 37 and recovering the polypeptide.

Embodiment 39. A method for preparing a cell or progeny thereof capable of expressing a polypeptide as defined in any of embodiments 1 to 25 comprising transforming/transfecting the cell with a vector of embodiment 33.

Embodiment 40. An isolated CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for use in medicine.

Embodiment 41. An isolated CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for use in therapy.

Embodiment 42. A pharmaceutical composition comprising the polypeptide as defined in any of embodiments 1 to 25 and a pharmaceutically acceptable carrier.

Embodiment 43. Use of an isolated CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the production of a medicament for the induction of tolerogenic dendritic cells in a subject.

Embodiment 44. Use of an isolated CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the production of a medicament for the treatment or prevention of one or more disease or disorders selected form the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft survival.
Embodiment 45. Use according to embodiment 44 for the treatment or prevention of autoimmune diseases.

Embodiment 46. Use according to embodiment 44 for the treatment or prevention of allergic hypersensitivity.

Embodiment 47. Use according to embodiment 44 for the prolongation of allograft survival in a subject in need thereof.

Embodiment 48. Use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the production of a medicament for the induction of tolerance of DC’s.

Embodiment 49. Use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the treatment or prevention of one or more diseases or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and myasthenia gravis.

Embodiment 50. The use according to embodiment 49, wherein said disease or disorder is autoimmune diseases.

Embodiment 51. The use according to embodiment 49, wherein said disease or disorder is allergic hypersensitivity.

Embodiment 52. A use according to embodiment 49 for prolongation of allograft survival in a subject in need of such.

Embodiment 53. The use according to any of embodiments 48 to 52, wherein the medicament comprising the DNA encoding the polypeptide is expressing the polypeptide in vivo.

Embodiment 54. Use of DNA encoding CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the generation of an agent, including siRNA, for the preparation of a medicament for reduced transcription or translation and thereby reduced expression of CD300LG protein in vivo after administration.

Embodiment 55. Use of DNA encoding CD300LG-ext polypeptide as defined in any of embodiments 1 to 25 for the generation of an agent, including siRNA, for the preparation of a medicament for the treatment or prevention of one or more diseases or disorders selected from the group consisting of autoimmune diseases, such as rheumatoid arthritis.
(RA), allergic hypersensitivity, asthma, inflammatory bowel disease (IBD) such as Morbus
Chron or colitis ulcerosa, systemic lupus erythematosus (SLE), psoriasis and other
dermatological disorders, multiple sclerosis (MS), insulin-dependent diabetes mellitus
(IDDM), and myasthenia gravis.

Embodiment 56. The use according to embodiment 55, wherein said disease or
order is autoimmune diseases.

Embodiment 57. The use according to embodiment 55, wherein said disease or
disorder is allergic hypersensitivity.

Embodiment 58. A use according to embodiment 55, for prolongation of allograft
survival in a subject in need of such.

Embodiment 59. An antibody which specifically recognizes the polypeptide as
defined in any of embodiments 1 to 25, or an antigen-binding fragment of said antibody.

Embodiment 60. An antibody according to embodiment 59, wherein said antibody is
a monoclonal antibody.

Embodiment 61. An antibody, according to embodiment 59 or embodiment 60,
wherein said antibody is a human antibody, a humanized antibody, or chimeric antibody.

Embodiment 62. An antibody according to any of embodiments 59 to 61, which
inhibits DC-dependent allogenic T cell proliferation as described in assays of example 6 and
7 and/or inhibits DC-T cell clustering as described in assay of example 8.

Embodiment 63. A pharmaceutical composition comprising the inhibitory antibody or
an inhibitory fragment thereof as defined in any of embodiments 59 to 62 and a
pharmaceutically acceptable carrier.

Embodiment 64. Use of an inhibitory antibody or an inhibitory fragment thereof as
defined in any of embodiments 59 to 62 for the production of a medicament for the induction
of tolerance of dendritic cells.

Embodiment 65. Use of an inhibitory antibody or an inhibitory fragment thereof as
defined in any of embodiments 59 to 62 for the production of a medicament for the treatment
or prevention of one or more disease or disorders selected from the group consisting of
autoimmune diseases, such as rheumatoid arthritis (RA), allergic hypersensitivity, asthma,
inflammatory bowel disease (IBD) such as Morbus Chron or colitis ulcerosa, systemic lupus
erythematosus (SLE), psoriasis and other dermatological disorders, multiple sclerosis (MS),
insulin-dependent diabetes mellitus (IDDM), myasthenia gravis, and prolongation of allograft
survival.

Embodiment 66. A use of an inhibitory antibody or an inhibitory fragment thereof as
defined in any of embodiments 59 to 62 for the preparation of a medicament for treating a
subject having a disease or disorder associated with increased CD300LG full-length expression.

Embodiment 67. An antibody according to any of embodiments embodiment 59 to 62, which activates DC-dependent allogenic T cell proliferation as described in assays of example 6 and 7 and/or activates DC-T cell clustering as described in assay of example 8.

Embodiment 68. A pharmaceutical composition comprising the activating antibody or an activating fragment thereof as defined in any of embodiments 59 to 62 and a pharmaceutically acceptable carrier.

Embodiment 69. Use of an activating antibody or an activating fragment thereof as defined in any of embodiments 59 to 62 for the production of a medicament for the reduction of tolerance of dendritic cells.

Embodiment 70. Use of an activating antibody or an activating fragment thereof as defined in any of embodiments 59 to 62 for the production of a medicament for the treatment or prevention of one or more disease or disorders selected from the group consisting of cancer or infections.

Embodiment 71. A use of an activating antibody or an activating fragment thereof as defined in any of embodiments 59 to 62 for the preparation of a medicament for treating a subject having a disease or disorder associated with reduced CD300LG full-length expression.

Embodiment 72. An assay method for in vitro determining the amount of CD300LG-FL in the tissues of a patient, preferably the method for determining tumor, autoimmune diseases, allergy, allograft survival, and infections, which comprises contacting a serum sample with the antibody or a CD300LG binding fragment thereof as defined in any of embodiments 59 to 62.

Embodiment 73. Variants and derivatives of CD300LG-ext that act as antagonists and/or agonists of the CD300LG receptor.

The features disclosed in the foregoing description may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.
EXAMPLES

Example 1
Identification of CD300LG as down-regulated by “tolerogenic” cytokines IL-21, IL-10, TNF-α, and TGF-β in murine dendritic cells.

CDNA microarray analyses
Bone marrow-derived DCs were generated as previously described (Brandt et al., 2003a; Lutz et al., 1999). At day 8, cells were removed by Accutase (PAA) and washed. Purity was routinely >95% as determined by FACS analysis of CD11c<sup>+</sup> expression. In vitro generated DCs were split into 6-well plates and left at rest for 2h. Subsequently, cells were treated for 24h with media control or 100ng/ml murine IL-21, 100ng/ml murine IL-10, 50ng/ml murine TNF-α, or 100ng/ml murine TGF-β, cytokine concentrations were chosen based on previous publications. Trizol was added for RNA isolation and lysates were frozen immediately at -80°C or cells were detached using Accutase and subsequently analyzed by FACS or in functional assays. Three individual experiments were performed.

Total RNA was isolated using TriZol (Invitrogen) followed by further purification on RNEasy columns (Qiagen) according to the guidelines provided by the manufacturers. 1.5µg RNA from each of the samples was amplified using the Aminoallyl messageAmp aRNA kit (Ambion) and labeled according to the protocol of the supplier. Cy3- and Cy5-labeled aRNAs from each of the biological replicates were hybridized to mouse inflammatory arrays (MWG) as well as “in-house arrays” spotted with a 20,000 gene-collection obtained from Lion Biosciences, according to the instructions of the vendors. Briefly, inflammatory arrays were pre-hybridized at 42°C for 45min in 4XSSC, 0.5% SDS, and 1% BSA, washed 5-times with H<sub>2</sub>O and dried. 120 pmol Cy3- and Cy5-labeled aRNA were mixed and fragmented to 60-200 nucleotides using RNA Fragmentation Reagents from Ambion according to their instructions. Fragmented nucleotides were subsequently purified on Microcon YM30 columns (Millipore) according to the protocol of the supplier, re-suspended in 30µl coverslip hybridization buffer (MWG), denatured at 95°C for 3min and hybridized in an ISO20 microarray hybridization incubator (Grants Instruments) at 42°C for 18h. Slides were then washed 5min at room temperature in 2XSSC, 0.1% SDS, 5min at room temperature in 1XSSC, and 5min at room temperature in 0.5XSSC, all washing buffers preheated to 30°C. Finally, slides were dried and scanned in an Axon4000B (Axon instruments). 3D-link slides (Amersham) were spotted with PCR products from mouse in-house 20,000 gene-collections (Lion Biosciences), fixed, and blocked as described by the vendor. Prior to hybridization, slides were pre-hybridized for 20min at 42°C in DIG easy hybridization buffer (Roche). 30pmol Cy3- and Cy5-labeled aRNA
were mixed, resuspended in 75 µl DIG easy hybridization buffer (Roche), denatured at 95°C for 2 min and hybridized in an ISO20 microarray hybridization incubator (Grants Instruments) at 42°C for 16 h. Slides were then washed twice in 2XSSC at room temp for 5 min, twice in 0.5XSSC, 0.1% SDS at 60°C for 15 min, rinsed in H2O and dried. Finally, slides were scanned in an Axon4000B (Axon instruments).

Spots on both types of arrays (Inflammatory (MWG) and Lion Biosciences) were automatically identified and signals as well as background signals surrounding the spots were measured by the use of GenePixPro software (Axon instruments).

Background smoothing was performed using a smoothing function of the following form:

\[
i_{pa} = \begin{cases} 
\frac{i_{fa} - i_{ba}}{\delta} & \text{if } i_{fa} - i_{ba} > \delta \\
\delta \exp[1 - (i_{ba} + \delta)/i_{fa}] & \text{otherwise}
\end{cases}
\]

(Edwards, 2003). The threshold values (\(\delta\)) were chosen in a slide/dye-specific fashion so that the proportion of spots with net intensities between 0 and \(\delta\) was 10% of the proportion with negative net intensities. For data from the inflammatory arrays, quantile normalization functions were computed on the basis of data from housekeeping genes only. The full data were then normalized using these functions (using linear interpolation between points of support). For data from the Lion arrays, first within-slide normalization (print-tip loess) was applied to adjust the log-intensities for spatial and dye bias. Then quantile normalization was applied to the log-intensities to adjust for between-slide differences. For each gene, a fixed effect ANOVA model including terms for spot effect and treatment effect was fitted to the normalized log-intensities. Estimates of the log-expression ratio comparing IL-21, IL-10, TNF-α, and TGF-β with media were calculated, together with the associated standard errors and t-tests for the hypothesis that the true log expression ratio is zero. Selection criteria for identification of genes regulated in common by all four “tolerogenic” cytokines were: p < 0.0001 and fold regulation > 2 or < 0.5 fold for at least one of the cytokines and regulation in the same direction for the remaining cytokines. CD300LG fulfilled these criteria.

Real time qPCR analyses

For validation of data obtained by cDNA microarray analyses, total RNA samples obtained from DCs isolated from three additional biological replicate experiments were reverse transcribed to cDNA using Superscript II reverse transcriptase (Gibco BRL) according to the manufacturer’s instructions. Expression of selected genes were determined using an ABI PRISM 7000 sequence detection system instrument and software (PE Applied Biosystems, Foster City, CA) as previously described (Gibson et al., 1996; Heid et al., 1996)
with minor modifications. Briefly, at least a five-point serial standard curve (each point performed in triplicate) was made using cDNA from DCs treated for 1h with media with the final assay concentration ranging from approximately 0.4-100ng total RNA/25 μl reaction. This curve was used to calculate the amount of target gene mRNA in all samples based on qPCR performed with approximately 25ng total RNA/25 ul. Universal PCR master mix and the following primer/probe assays were used: Mm01266005_m1 (Cd300lg) according to the instructions of the supplier (Applied Biosystems). mRNA expression of Cd300lg was normalized to the expression level of acidic ribosomal phosphoprotein PO (36B4) (Mm00725448_m1). A t-test was used to determine statistical significance (see Figure 1).

Example 2
Recombinant expression of the extracellular domain of human CD300LG fused to mFc in Hek293 6E cells.

Cloning

The extracellular part of hCD300LG (hCD300LG-ext), including potential signal peptide, are PCR amplified from reverse transcribed cDNA from human Quantitative Human Reference Total RNA (Clontech) using the Phusion Taq polymerase and the following primers: hCD300LGEtxfwd42Mam: 5'-GAATTCGCCACCATGCGGTCTCTGCTCTGCTA-3' and hCD300LGEtxrev783: 5'-CTCGAGGCGGGACCATCAGGATGGACA-3'. Parameters for PCR are: 1) initial denaturation at 98 °C for 30 s; 2) touchdown PCR including 6 cycles of denaturing at 98 °C for 5 s, annealing at 61 down to 56 °C for 10 s, extension at 72 °C for 30 s, 3) 30 cycles of denaturing at 98 °C for 5 s, annealing at 55 °C for 10 s, extension at 72 °C for 30 s. The amplified PCR fragments are cloned into zero blunt (Invitrogen) and correct nucleotide sequence are verified by sequencing (MWG). The extracellular domains of CD300LG (hCD300LG-ext) is excised from zero blunt with EcoRI and Xhol and cloned into the pcDNA 3.1 vector containing the Fc part of murine IgG1 (amino acid 98-324 of image clone BC002121), resulting in hCD300LG-ext-mFc.

Expression

The plasmids containing hCD300LG-ext-mFc are transfected into Hek293 6E cells by transient transfection. Hek293 6E cells adapted to suspension culture in Freestyle 293 expression medium (Invitrogen), supplemented with 25 ug/ml Geneticin, 0.1% Pluronic F-68 (Sigma), and 1% pencilllin/streptomycin (Durocher et al., 2002). Cells are cultured in Erlenmeyer flasks using 15-25% of the nominal volume at 100-130 r.p.m. under standard humidified conditions (37 °C and 8% CO2). Three hours before transfection, cells are centrifuged and resuspended in fresh medium supplemented with 25 ug/ml Geneticin, 0.1%
Pluronic F-68 (Sigma), and 1% pencillin/streptomycin (Invitrogen) at a density of 1 x 10^6 cells/ml. 500 ug plasmid/l of cells are diluted in OPTI-MEM I medium (Invitrogen) to a final volume of 33 ml. Similarly, 1320 ul transfectin reagent (Invitrogen)/l cells is diluted in in OPTI-MEM I medium (Invitrogen) to a final volume of 33 ml and left for 5 min. at room temp.

Plasmid diluted in OPTI-MEM I and transfectin diluted in OPTI-MEM I are mixed and incubated at room temp for 20 min, after which the mixture is added to the cells. After three days of shaking Glutamax I (Invitrogen) is added and cells are left for shaking additional 4 days. At day 7, cells are centrifuged and supernatants are sterile filtered and hCD300LGext-mFc fusion proteins purified as described below.

**Example 3**

Recombinant expression of the extracellular domain of murine and human CD300LG (mCD300LGext and hCD300LGext) fused to a his-tag in Escherichia coli.

**Cloning**

The extracellular domains CD300LG from mouse (mCD300LG-ext-his) and human (hCD300LG-ext-his), excluding potential signal peptides, and including 6 histidine residues C-terminally, are PCR amplified from reverse transcribed cDNA from mouse and human Quantitative Human Reference Total RNA (Clontech), respectively, using the following primers: mouse: mCD300LGfwd98Bact: 5’-CTATGCTGAAGGGTCCAAAAGGAGATC-3’ and mCD300LGrev523 5’-

hCD300LGfwdhis96Bact: 5’-CATATGCTGAGGGGCCAGAGAAATC-3’ and hCD300LGrrev782: 5’-CTCGAGGCGGCCATCCGGATGACAC-3’. Parameters for PCR are: 1) initial denaturation at 98 °C for 30 s, 2) touchdown PCR including 6 cycles of denaturing at 98 °C for 5 s, annealing at 61 down to 56 °C for 10 s, extension at 72 °C for 30 s, 3) 30 cycles of denaturing at 98 °C for 5 s, annealing at 55 °C for 10 s, extension at 72 °C for 30 s. The amplified PCR fragments are cloned into zero blunt (Invitrogen) and correct nucleotide sequence verified by sequencing (MWG). The extracellular domains of CD300LG are excised from zero blunt and cloned into the pET20b+ vector.

**Expression**

The plasmids containing mCD300LG-ext-his and hCD300LG-ext-his are transformed into an E. coli strain, which subsequently can be used for expression of the proteins encoded by the plasmids by a fermentation process.
The fermentation process consists of a one-step propagation on a solid agar surface followed by a fed-batch fermentation using glycerol feeding according to a predefined feeding profile. After 16-18 hours of elapsed fermentation time (OD₉₀₀ ≥50), IPTG is added to induce expression of mouse or human CD300LGext-6xhis. Approximately 6-8 hours after induction, the cells are harvested by centrifugation.

Preparation of the inoculum is done using a single-step propagation on solid LB-agar. Prior to inoculation, ampicillin is added to the flask, giving a final concentration of 100 mg/l. Ampicillin is added by spreading 1-2 ml of a filter-sterilised stock solution of ampicillin uniformly out over the agar surface, allowing the ampicillin to diffuse into the agar. The agar flask is inoculated with 100 µl of the cell bank vial and incubated at 37°C for 6-8 hours. The inoculum is harvested by suspending the cells in 5 ml of sterile 0.9% NaCl solution. The OD₉₀₀ of the suspension should be >5. The fermenter is inoculated with cells corresponding to a start OD₉₀₀ of 0.005 – 0.015 in the fermenter.

The fermentation medium composition is given in the table below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>40</td>
<td>g/l</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>3.3</td>
<td>g/l</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>3.3</td>
<td>g/l</td>
</tr>
<tr>
<td>Pluronic</td>
<td>0.5</td>
<td>ml/l</td>
</tr>
<tr>
<td>Glycerol (87%)</td>
<td>15</td>
<td>g/l</td>
</tr>
<tr>
<td>#MgSO₄.7H₂O (1M stock)</td>
<td>5.3</td>
<td>ml/l</td>
</tr>
<tr>
<td>#Trace element solution</td>
<td>34</td>
<td>ml/l</td>
</tr>
<tr>
<td>#Ampicillin</td>
<td>100</td>
<td>mg/l</td>
</tr>
</tbody>
</table>

# added after autoclaving

During fermentation, the protocol follows a predefined feeding profile aiming at glycerol-limited growth. The start working volume of the fermentation is 3 L. The fed-batch phase is started after 9 hours by feeding the glycerol feed solution (718 g/l) at a rate of 0.25 g/min. The feed rate is increased linearly to reach 0.5 g/min after 13 hours, and further to 0.7 g/min after 17 hours, whereafter the feed-rate is kept constant until the end of the fermentation. Dissolved oxygen tension (DOT) is kept above 20% air saturation by increasing stirrer speed. Gassing is performed using air at a flowrate of 1.5 vvm. Under these conditions, off-gas analysis will peak between 4-8% of CO₂. Throughout the fermentation, pH is kept at 6.9 by titration of 10% H₃PO₄ and 10% NH₄OH. Due to the nature of the medium,
there will be distinct phases of acid and base titration. Growth on glycerol promotes a drop in pH while consumption of amino acids/peptides from the yeast extract will lead to a rise in pH.

Induction is carried out by addition of IPTG at a final concentration of 1 mM. The time for IPTG addition is 16-18 hours elapsed fermentation time, at an OD$_{600}$ of at least 50. The duration of the induction period is 6-8 hours. The fermentation is stopped after 22-24 hours. The final OD$_{600}$ is expected to be approximately 110 ±20. The cells are harvested by centrifugation (4500 rpm, 20-40 min, 4°C). The cell pellets are stored at -80°C prior to further purification.

Example 4

Purification of recombinant extracellular domain of human CD300LG fused to a mFc expressed in Hek293 6E cells

Purification of CD300LG-mFc-fusion proteins is done using an ÄKTA Explorer system (Amersham Biosciences). A XK16 column is filled with 10 ml of Streamline rProtein A (Amersham Biosciences) and equilibrated with PBS-buffer, pH 7.4 (10 column volumes).

Supernatant from transfected Hek 293 6E cells containing the mFc-fusion protein is loaded onto the Streamline rProtein A column with a flow rate of 5 ml/min. Unbound proteins are removed from the column with 3 volumes wash in PBS pH 7.4. Bound mFc-fusion proteins are eluted from the column with 50 mM glycine-buffer pH-adjusted to pH 3.0 with HCl with a flow rate of 1 ml/min. To prevent protein precipitations at low pH values, fractions of 3 ml are collected in tubes containing 3 ml of PBS pH 7.4. Fractions containing the mFc-fusion protein are pooled and filtered in a stirred Amicon cell with an ultrafiltration membrane, NMWL 10.000 (Amicon, Millipore Corporation). A 16/60 Superdex 200 column (Amersham Biosciences) is equilibrated with PBS-buffer pH 7.4 with a flow rate of 2 ml/min. Then 10 ml of the concentrated mFc-protein sample is applied to the column with a flow rate of 1 ml/min. Fractions of 3 ml are collected and the peak fractions pooled and filtered by centrifugation in Centriprep Ultracel YM-10 MWCO 10.000 (Amicon, Millipore Corporation).

Example 5

Purification of recombinant extracellular domain of mouse and human CD300LG fused to a his-tag expressed in Escherichia coli.

E. coli pellet is re-suspended in a lysis-buffer containing 6 mg/ml PBS buffer pH 7.4, complete tablets (3 tablets in 500 ml) (Roche Diagnostics, Mannheim Germany) and DNase (5 mg/ml) and stirred for 15 min at 10°C. The cell suspension is loaded onto a cell disruptor (2 times 1500-2000 bar). When cells are bust the suspension is spun for 1. hour at 38,700 g.
The supernatant is loaded onto a 20 ml Ni-NTA-column (QIAGEN GmbH, Hilden, Germany) using an ÄKTA Explorer system (Amersham Biosciences) with a flow of 3.00 ml/min. The column is washed in Buffer A: 5 mM imidazole, 300 mM NaCl, 10 % glycerol pH 7.8 and eluted 10 % stepwise in Buffer B: 300 mM imidazole, 300 mM NaCl, 10 % glycerol pH 7.8. Fractions of 10 ml are collected.

Fractions are analyzed by SDS-PAGE and western blotting. Fractions containing CD300LG ext-his polypeptides are pooled and loaded onto a MonoQ column (GE Healthcare, Uppsala, Sweden). The column is washed in Buffer A: 20 mM Tris pH 8.0 and eluted with Buffer B: 20 mM Tris, 1 M NaCl pH 8.0 with a flow of 1 ml/min. Fractions of 2 ml are collected and tested by SDS-PAGE and Western blotting. Fractions containing CD300LG ext-his polypeptides are pooled and filtered on Centriprep Ultracel YM-10 MWCO 10.000 (Amicon, Millipore Corporation, Bedford, U.S.A.).

To separate potential CD300LG ext-his monomers, dimers and multimers a 26/60 Superdex 75 column (GE Healthcare, Uppsala, Sweden) is equilibrated with PBS-buffer pH 7.4 with a flow rate of 2 ml/min. Then the concentrated CD300LG ext-his samples are applied to the column with a flow rate of 2 ml/min. Fractions of 3 ml are collected and investigated by SDS-PAGE. Fractions containing CD300LG ext-his potential monomers, dimers and multimers are pooled individually. The poole is filtered by centrifugation in Centriprep Ultracel YM-10 MWCO 10.000 (Amicon, Millipore Corporation, Bedford, U.S.A.).

Example 6

Mixed lymphocyte reaction (MLR) assays (human).

Unless otherwise noted, all cells are cultured using a standard medium (1% human plasma medium), which consists of RPMI 1640 (BioWhittaker) supplemented with glutamine (200 µg/ml) (BioWhittaker), penicillin/streptomycin (20 µg/ml), 10 mM Hepes, pH 7.5 (Sigma-Aldrich), and 1% heat-inactivated (56°C; 30 min) human plasma from a single donor.

Generation of Dendritic Cells (DCs)

PBMCs are isolated from buffy coats by sedimentation in Ficoll-hypaque (Amersham Pharmacia Biotech) and seeded onto IgG-coated (10 µg/ml γ-globulin from Cohn fraction; Sigma-Aldrich) 100 mm-culture dishes and incubated at 37°C in 5 % CO2. After 1 and 7 h incubations, non-adherent cell fractions are harvested, and the adherent cells are further cultured in 1% human plasma medium supplemented with the cytokines GM-CSF (800 U/ml) and IL-4 (500 U/ml). Fresh medium with GM-CSF to a final concentration of 400 U/ml and IL-4 (500 U/ml) is added on day 3 of the incubation period. On day 4 or 5, non-adherent cells are collected, counted, and transferred into new dishes at a density of 0.3-0.5 x 105 cells/ml.
For final DC maturation, 1% human plasma medium was supplemented with TNF-α, (1.25 ng/ml), GM-CSF (40 U/ml), IL-4 (200 U/ml), prostaglandin E2 (0.5 μg/ml). (Lechmann, M. et al. (2001) J.Exp. Med. 194: 1813-1821).

**Allogenic MLR**

CD4+ and CD84+ T cells are isolated from buffy coats (harvested non-adherent cell fractions are incubated with neuramidase treated sheep erythrocytes, collected by ficoll gradient centrifugation and cultured in RPMI, supplemented with 5% human serum from a single donor) and stimulated with different ratios of mature allogenic DCs. The cells are left untreated or are incubated with different concentrations of CD300LGext or with control protein. T-cells (2 x 10^5 / well) and DCs are co-cultivated for 4 days in 200 μl RPMI, supplemented with 5 % human serum from a single donor in 96-well cell culture dishes. Cells are pulsed with [3H] -thymidine (1 μCi/well; Amersham Pharmacia Biotech) for 16 h. The culture supernatants are harvested onto glass fiber filtermates using an IH-110 harvester (Inotech, Dottikon, Switzerland), and filters are counted in a 1450 microplate counter (Wallac, Turku, Finland) (Lechmann, M. et al. (2001) J.Exp. Med. 194: 1813-1821).

**Example 7**

**Mixed lymphocyte reaction (MLR) assays (murine).**

Male or female C57/BL6 mice and BALB/C mice are used at the ages of between 1 and 4 months.

**Generation of bone marrow (BM)-DCs**

The generation of BM-DCs from C67/BL6 mice is performed exactly as described (J. Immunol. Methods 223:77, 1999). RPMI 1640 (Life Technologies) is supplemented with 100 U/ml penicillin (Sigma), 100 μg/ml streptomycin (Sigma), 2 mM L-glutamine (Sigma), 50 μg/ml ME (Sigma), 10 % heat- inactivated filtered FCS (InVitrogen). GM-CSF is used at 200 U/ml (PrepTech/Tebu, Rocky Hill, NJ) on days 0, 3, 6 and 8 of incubation period.

**Allogenic MLR**

CD4+ and CD8+ T cells are isolated from inguinal and mesentachymal lymph nodes of BALB/C mice and used for the allogenic MLR. These T-cells (2x205 cells/well) and day 9 BM-DCs (at different ratios) are co-cultured for 3 days in 200 μl RPMI 1640 supplemented with 100 U/ml penicillin, 100 (μg/ml streptomycin, 2 mM L-glutamine, 50 μg/ml ME, 10 % heat- inactivated filtered FCS in 96-well cell culture dishes. Cells are pulsed with [3H] -thymidine (1 μCi/well; Amersham Pharmacia Biotech) for 16h. The culture supernatants are harvested onto glass fiber filtermates using an IH-110 harvester (Inotech, Dottikon,
Switzerland), and filters were counted in a 1450 microplate counter (Wallac, Turku, Finland).

**Example 8**

**DC-T-cell cluster formation**

T cells (2×10^5/well) are cultured in 100 μl RPMI 1640/5% HS AB per well in 96 well cell culture plates. Mature DC (day 7–8) are added in graded numbers to the T cell cultures. T cells or DC cultured alone are used as negative controls. To investigate the effect of CD300LG-ext polypeptides, CD300LG-ext polypeptides are added to the cultures. The cells are then co-cultured for 4 days. During this time the cluster formation is analysed daily under the microscope. Coverslips are coated for 30 min with Poly (L)-Lysine (0.1 mg/ml; Sigma-Aldrich) and incubated at room temperature. After washing, coverslips are put in 12 well plates and 2×10^5 cells (2×10^5 cells/ml medium) are added and incubated at 37°C in 5% CO₂. After 30 min when cells have settled they are fixed with 2 ml 3% paraformaldehyde (PFA) in PBS for 30 min at room temperature followed by 3 washes. To quench the fluorescence of the PFA 100 mM Glycine/PBS is added for 10 min at room temperature. To permeabilize the membranes, the cells are treated for 4 min with 0.1% Triton X-100 (Boehringer Manheim) in PBS. After two washes cells are blocked with PBS/1% BSA for 30 min at room temperature. For the staining, Fascin- (Dako) and Tubulin-specific antibodies (MoBiTec) are added for 30 min at room temperature and then washed 3×5 min with PBS. Cells are then incubated with goat-anti-mouse-Alexa Fluor 488-labelled antibody (MoBiTec) for 30 min at RT in the dark. For the phalloidin staining a directly FITC-conjugated antibody (Sigma-Aldrich) is used. Preparations are washed again 3×5 min with PBS. The cover slips are mounted using 7 μl of Mowiol (Calbiochem-Merck) onto the slides and dried overnight at room temperature. The pictures are taken using a Leica DMRB microscope (Leica) and analysed.
CLAIMS

1. An isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-247 of SEQ ID No. 4, a variant, a splice variant, a fragment or a derivative thereof.

2. A CD300LG-ext polypeptide according to claim 1 having an amino acid sequence, which sequence has at least 90% identity to the amino acid sequence of amino acids 19-247 of SEQ ID No. 4.

3. An isolated CD300LG-ext polypeptide having the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6, a variant, a splice variant, a fragment or derivative thereof.

4. A CD300LG-ext polypeptide according to claim 3 having an amino acid sequence, which sequence has at least 90% identity to the amino acid sequence of amino acids 19-162 of SEQ ID No. 5 or 6.

5. A CD300LG-ext polypeptide according to any of claims 1 to 4, which inhibits DC-dependent allogenic T cell proliferation as described in assays of example 6 and 7 and/or inhibits DC-T cell clustering as described in assay of example 8.

6. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide of any of claims 1 to 5.

7. A nucleic acid molecule according to claim 6 having a nucleotide sequence of 42-782 of SEQ ID No. 1.

8. A vector containing a nucleic acid molecule of claim 7.

9. A host cell comprising the vector of claim 8.

10. A method for producing a polypeptide as defined in any of claims 1 to 5 comprising expressing the polypeptide encoded by the nucleic acid molecule of the host cell of claim 9 and recovering the polypeptide.

11. An isolated CD300LG-ext polypeptide as defined in any of claims 1 to 5 for use in therapy.
12. A pharmaceutical composition comprising the polypeptide as defined in any of claims 1 to 5 and a pharmaceutically acceptable carrier.

13. Use of an isolated CD300LG-ext polypeptide as defined in any of claims 1 to 5 for the production of a medicament for the induction of tolerogenic dendritic cells in a subject.

14. Use of an isolated DNA nucleic acid molecule or vector comprising a nucleotide sequence encoding a CD300LG-ext polypeptide as defined in any of claims 1 to 5 for the production of a medicament for the induction of tolerance of DC’s.

15. Use of DNA encoding CD300LG-ext polypeptide as defined in any of claims 1 to 5 for the generation of an agent, including siRNA, for the preparation of a medicament for reduced transcription or translation and thereby reduced expression of CD300LG protein in vivo after administration.

16. An antibody which specifically recognizes the polypeptide as defined in any of claims 1 to 5, or an antigen-binding fragment of said antibody.

17. Use of an inhibitory antibody or an inhibitory fragment thereof as defined in claim 16 for the production of a medicament for the induction of tolerance of dendritic cells.

18. An assay method for in vitro determining the amount of CD300LG-FL in the tissues of a patient, preferably the method for determining tumor, autoimmune diseases, allergy, allograft survival, and infections, which comprises contacting a serum sample with the antibody or a CD300LG binding fragment thereof as defined in claim 16.

19. Variants and derivatives of CD300LG-ext that act as antagonists and/or agonists of the CD300LG receptor.
Fig. 2

CD300LG

TREM4α

TREM4β

Ig-like
domain

Sign pep

Ext domain

TM

Int domain

1 126 212 247 269 295 332

1 126 Ext domain Ext domain Int domain

1 127 Ext domain Ext domain Int domain

1 127 Ext domain Ext domain Int domain

1 18 184 127 184 195 222