Title: METHOD OF TREATMENT OF IMMUNOLOGICAL DISEASES

Abstract: A method for treating or preventing a disease capable of being alleviated by interleukin 10 (IL-10) and/or IL-4 in a subject in need, comprising administering to the subject a therapeutically effective amount of a thymus polypeptide. Examples of such diseases include infectious diseases, acute and chronic inflammatory diseases, cancer, transplantation rejection and autoimmune diseases. Also disclosed is a pharmaceutical composition comprising the thymus polypeptide for treating those diseases.
METHOD OF TREATMENT OF IMMUNOLOGICAL DISEASES

FIELD OF THE INVENTION

This invention relates to the treatment of diseases having an immunological component.

PRIOR ART

The following is a list of prior art, which is considered to be pertinent for describing the state of the art in the field of the invention. Acknowledgement of these references herein will be made by indicating the number from their list below within brackets.


BACKGROUND OF THE INVENTION

Interleukin-10 (IL-10) is a homodimeric cytokine which has potent anti-inflammatory and immunosuppressive effects, especially on monocytes, dendritic cells and macrophages \(^{(1)}\). For example, a single, systemic administration of an adenoviral vector encoding viral IL-10 was found to inhibit murine collagen-induced arthritis \(^{(2)}\). Type I diabetes was prevented in nonobese diabetic mice by early systemic treatment with high doses of a vector expressing murine IL-10 \(^{(3)}\). Intra-peritoneal administration of adenoviral IL-10 to mice significantly reversed colitis induced by administration of DSS \(^{(4)}\).

It is therefore to be expected that modulation of IL-10 expression will have an effect on those diseases that have an immunological component or etiology such as infectious diseases, acute and chronic inflammatory diseases, cancer, transplantation and autoimmune diseases\(^{(5)}\).

Autoimmune diseases are mediated by primarily cellular or humoral immune components. Among the autoimmune diseases mediated primarily by cellular components are multiple sclerosis (MS), autoimmune uveitis, autoimmune uveoretinitis, autoimmune thyroiditis, Hashimoto's disease, insulitis, Sjogren's syndrome, experimental autoimmune myocarditis, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Crohn's disease, and diabetes type I.

Among the various cells involved in the immune response, two different types of T-cells have been identified. TH1 cells secrete the cytokines interleukin 2 (IL-2), interferon-\(\gamma\) (IFN-\(\gamma\)) and tumor necrosis factor \(\text{\(\alpha\)}\) (TNF\(\text{\(\alpha\)}\), and induce the cell-mediated (delayed-type hypersensitivity) immune response. TH2 cells secrete the cytokines IL-1\(\alpha\), IL-4, IL-5 and IL-10, and induce the humoral (antibody) immune response. Furthermore, it has been found that IL-10 inhibits antigen-presenting cells (APC) such as macrophages which activate TH1 cells, while IFN-\(\gamma\) inhibits the production of cytokines by TH2 cells. In particular, IL-10 has an immunosuppressive effect and inhibits the production of pro-inflammatory mediators involved in cell-mediated autoimmune disease \(^{(2)}\). IL-4 has also been used to treat autoimmune diseases \(^{(7)(8)(9)}\).

Recently, a cDNA unique for the human thymus was identified. The peptide encoded by the cDNA has been named TH10L. The peptide is described in copending
SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for treating diseases which may be alleviated by IL-10 and/or IL-4.

In a first aspect of the invention, there is provided a method for treating or preventing a disease capable of being alleviated by interleukin 10 (IL-10) and/or by IL-4 in a subject in need, comprising administering to the subject a therapeutically effective amount of an isolated polypeptide from the group consisting of:

(a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

(b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1.

The term "a disease which may be alleviated by IL-10 and/or IL-4" is used herein to denote diseases having an immunological component or etiology which may be treated either by administration of IL-10 and/or of IL-4 or by inducing the endogenous production of IL-10 and/or of IL-4. Examples of such diseases include infectious diseases, acute and chronic inflammatory diseases, cancer diseases, transplantation rejection and autoimmune diseases.

Infectious diseases which may be treated in accordance with the invention include diseases resulting from bacterial, fungal or protozoan infections.

Examples of inflammatory diseases which may be treated in accordance with the invention include sepsis, endotoxemia, pancreatitis, uveitis, hepatitis, peritonitis, keratitis, SIRS and injury-induced inflammation.
Examples of autoimmune diseases which may be treated in accordance with the invention include multiple sclerosis (MS), autoimmune uveitis, autoimmune uveoretinitis, autoimmune thyroiditis, Hashimoto's disease, insulitis, Sjogren's syndrome, spontaneous abortions, experimental autoimmune myocarditis, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Crohn's disease, lupus (SLE), psoriasis and diabetes, particularly type I.

Additional examples of autoimmune diseases which may be treated in accordance with the invention include Acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Allergic asthma, Allergic rhinitis, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome (APS), Autoimmune aplastic anemia, Autoimmune dysautonomia, Autoimmune hepatitis, Autoimmune hyperlipidemia, Autoimmune immunodeficiency, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune thrombocytopenic purpura (ATP), Axonal & neuronal neuropathies, Bal's disease, Behnet's disease, Bullous pemphigoid, Cardiomyopathy, Castleman disease, Celiac sprue (nontropical), Chagas' disease, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatomyositis, Devic disease, Discoid lupus, Dressier's syndrome, Endometriosis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evan's syndrome, Fibromyalgia**, fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia , Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, Immunoregulatory lipoproteins, Inclusion body myositis, Insulin-dependent diabetes (type I), Interstitial cystitis, Juvenile arthritis, Juvenile diabetes, Kawasaki syndrome, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lyme disease, Meniere's disease, Microscopic polyangiitis, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Myasthenia gravis, Myositis, Narcolepsy, Neutropenia, Ocular cicatricial pemphigoid, Osteoarthritis, Palindromic
rheumatism, Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis, Psoriatic arthritis, Idiopathic pulmonary fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynaud's phenomenon, Reflex sympathetic dystrophy, Reiter's syndrome, Relapsing polychondritis, Restless legs syndrome, Rheumatic fever, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis (SBE), Sympathetic ophthalmia, Takayas's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura (TTP), Autoimmune thyroid disease, Tolosa-Hunt syndrome, Transverse myelitis & necrotizing myelopathy, Ulcerative colitis, Undifferentiated connective tissue disease (UCTD), Vasculitis, Vesiculobullous dermatosis, Vitiligo and Wegener's granulomatosis.

In the present specification, the term "amino acid" will generally refer to an L-amino acid, unless specifically indicated otherwise.

In one embodiment of the invention, the polypeptide consists of the following sequence (SEQ ID. NO: 1):

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LHLWLSGEPVQSSGTMRSKSDSKRVSDKQLISKAVWWT
FFLPSTLWERK (SEQ. ID. NO: 1)
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This polypeptide will be referred to herein as the "T101 peptide " or "TIOF. The term "peptide " is used herein to denote a peptide, polypeptide or protein. The peptide may be obtained synthetically, through genetic engineering methods, expression in a host cell, or through any other suitable means.

A nucleic acid molecule comprising a sequence encoding for the TIOI peptide includes the following sequence (SEQ. ID. NO: T):

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CATCTCTGGCTTAGTGGGGAGCCAGTCCAGAGCTCTGGAACAAA
GGACATGAGATCCAAATCCGATTCCAAGCGAGTGAGTGACAAGC
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AGCTAATTTCCAAAGCTGTGTGGGACATTTTTTCTTCCTTCAA
CCCTCTGGGAGAGAAAATGA (SEQ. ID. NO: 2)

The TlOl peptide is included in a larger polypeptide encoded by a cDNA which is 84 amino acids long and includes a signal peptide of 33 amino acids on its N-terminal end. The cDNA sequence (SEQ. ID. NO: 3) and amino acid sequence (SEQ. ID. NO: 4) of this longer peptide are as follows:

ATGATGGCACTCAGAAGCCAGGGGCTCATGTTACCCCAGA
GCTGCCACAGACGGGCTCTTTCTCACCCTAACGTTAAGGCGGTG
AGCAGTGTCTTTTCAAGCCTCTGATCTCCTGATTAGGG
GAGCCAGTCCAGAGCTCTGGAACAAAGGACATGAGATCCA
AATCCGATTCCAAGCGATGAGTGACAAGCAGCTAATTTC
CAAAGCTGTGTGGTGGACATTTTTTCTTCCTTCAACCCTCTGGGAGAGAAAATGA

(SEQ. ID. NO: 3)

MMALRSQGLMLPQSCPQLAFLTLSALAAVSFSALHLWLSSG
EPVQSSGTKDMRSKDSKRSVSDKQLISKAVWWTFFLPSTL
WERK (SEQ. ID. NO: 4)

The polypeptide of SEQ. ID. NO: 3 will be referred to herein as the "full TlOl peptide". The full TlOl peptide may also be used in the method of the invention.

The term "active ingredient" may be used at times in the specification to denote the active substance used in the method of the invention, such as TlOl or a derivative thereof.

In a second embodiment of the invention, the polypeptide consists of an amino acid sequence of SEQ. ID. NO: 1 or SEQ. ID. NO: 4, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule.

The term "biological characteristics", with respect to a peptide molecule, refers to the peptide's ability to exert at least one of the in vitro or in vivo effects that may be exerted by the TlOl peptide or the full TlOl peptide, including but not limited to the biological activities reported below in the Examples. The term "biological
characteristics", with respect to a nucleic acid molecule, refers to the property of encoding a peptide having similar biological characteristics to that of the TIOI peptide or the full TIOI peptide, including, in particular: (i) a nucleic acid molecule that has a different sequence to that of SEQ. ID. NO: 2 or SEQ. ID. NO: 3, but, owing to the redundancy of the genetic code, encodes the TIOI peptide or the full TIOI peptide, respectively; and (ii) a nucleic acid molecule that encodes an amino acid molecule with a different sequence than that of the TIOI peptide or the full TIOI peptide but that has similar biological characteristics to that of the TIOI peptide or the full TIOI peptide, respectively.

The term "without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule" means to denote that the modified molecule retains a biological activity qualitatively similar to that of the unmodified molecule. With respect to a modified peptide, this means that it retains one or more of the biological characteristics of a peptide of SEQ. ID. NO: 1 or SEQ. ID. NO: 4, including, among others, its therapeutic utilities, as specified in this specification, as well as its in vitro and in vivo activities reported in the Examples below. In order to determine whether a peptide retains a biological activity qualitatively similar to that of the unmodified molecule, one or more assays can be carried out, such as for example an in vitro, in vivo or a clinical experiment in which a modified peptide is compared to the corresponding unmodified one (namely that of the TIOI peptide or the full TIOI peptide) that is assayed in parallel; or an experiment in which the modified peptide is assayed to examine whether it has a biological effect similar to that of the unmodified peptide as known from separately conducted experiments. Such an experiment may be carried out, for example, in a manner described in the Examples below. With respect to a modified nucleic acid molecule, the term "without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule" denotes the property of encoding a modified peptide of any of the above characteristics.

In a third embodiment of the invention, the polypeptide consists of a peptide comprising a partial contiguous sequence from the TIOI peptide including at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said TIOI peptide. Such a peptide will be referred to herein as a "partial TIOI peptide."
In a fourth embodiment of the invention, the polypeptide consists of a partial TIOI peptide that comprises a contiguous sequence of 13 amino acid residues beginning from the N-terminal end of the TIOI peptide (amino acid nos. 39 to 51), as follows:

**WTFFLPSTLWERK** (SEQ. ID. NO: 5),

and will be referred to herein as the "**13aa TIOI peptide**".

A partial TIOI peptide may be a peptide that includes a contiguous sequence of at least 8, 12, 15, 20, 25, 30, 35, 40 or at least 45 amino acid residues that has a degree of identity to a corresponding sequence of at least 8, 12, 15, 20, 25, 30, 35, 40 or at least 45 amino acid residues included in the TIOI peptide, the degree of identity being at least 70%, preferably at least 80%, more preferably at least 90% and particularly at least 95%.

A protein or polypeptide comprising an amino acid sequence of the full TIOI peptide, TIOI peptide, modified peptide or a partial TIOI peptide (such protein or polypeptide will be referred to herein as "**TIOI comprising protein**") may also be used in the method of the invention. The TIOI comprising protein may, for example, be a fusion protein that comprises the full TIOI peptide, the TIOI peptide, a modified peptide or a partial TIOI peptide; it may be a conjugate of a protein or another peptide or polypeptide with the full TIOI peptide, TIOI peptide, modified peptide or partial TIOI peptide; etc.

Additional partial TIOI peptides which may be used in the method of the invention are as follows:

- **SGEPVQSSGKDMRSKDSKRVS** (SEQ. ID. NO: 6)
- **DKQLISKAVWWTFFLPSTLWERK** (SEQ. ID. NO: 7)
- **PSTLWERK** (SEQ. ID. NO: 8)
- **AVWWTFFLPSTLW** (SEQ. ID. NO: 9)
- **KREWLTSPLFFTWWVA** (SEQ. ID. NO: 10)
- **WTFFL** (SEQ. ID. NO: 11)

SEQ. ID. NO: 6 consists of amino acids 6 to 28 of the TIOI peptide; SEQ. ID. NO: 7 consists of amino acids 29 to 51 of the TIOI peptide; SEQ. ID. NO: 8 consists of
amino acids 44 to 51 of the TIOI peptide; SEQ. ID. NO: 9 consists of amino acids 36 to 48 of the TIOI peptide; SEQ. ID. NO: 10 consists of amino acids 36 to 51 of the TIOI peptide in the reverse order; and SEQ. ID. NO: 11 consists of amino acids 39 to 43 of the TIOI peptide.

Further examples of partial TIOI peptides are modified peptides derived from any of the peptides defined above, e.g., modified peptides in which one or more amino acids are replaced by another amino acid by conservative substitution. As used herein, "conservative substitution" refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature. Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (He, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu is a conservative substitution.

In one embodiment, only one substitution is made in the amino acid sequence. In another embodiment, two substitutions are made. In a further embodiment, three substitutions are made. The maximum number of substitutions should not exceed that number of amino acids which leaves at least 70%, desirably at least 80%, preferably at least 90%, most preferably at least 95% of the amino acids in the unsubstituted sequence. By one preferred embodiment, the substitutions which include up to 3, at times up to 6 amino acid residues substituted by others, are conservative substitutions.

In a further embodiment, one or more amino acids of the peptides, proteins or polypeptides of the invention may be replaced by D-amino acids, preferably the corresponding D-amino acids.

In a still further embodiment, sequences of the reverse order of the above sequences may also be used in the invention.

Thus, full TIOI peptides of SEQ ID NO: 4 or preferably TIOI peptides of SEQ ID NO: 1 or partial TIOI sequences thereof, modified by one or more conservative substitutions may also be used in the method of the invention.

These peptides include at least 10, or 15, or 20, or 25, or 30, or 35, or 40 amino acid residues, or the entire sequence of the TIOI peptide having the sequence: AA_i,-AA_{i-1}-...-AA_{j}, wherein:
AA₁ is selected from leucine, isoleucine, valine and methionine;
AA₂ is selected from lysine, arginine and histidine;
AA₃ is selected from leucine, isoleucine, valine and methionine;
AA₄ is selected from tryptophan, phenylalanine and tyrosine;
AA₅ is selected from leucine, isoleucine, valine and methionine;
AA₆ is selected from serine, threonine, alanine, glycine and proline;
AA₇ is selected from serine, threonine, alanine, glycine and proline;
AA₈ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₉ is selected from serine, threonine, alanine, glycine and proline;
AA₁₀ is selected from leucine, isoleucine, valine and methionine;
AA₁₁ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₁₂ is selected from serine, threonine, alanine, glycine and proline;
AA₁₃ is selected from serine, threonine, alanine, glycine and proline;
AA₁₄ is selected from serine, threonine, alanine, glycine and proline;
AA₁₅ is selected from serine, threonine, alanine, glycine and proline;
AA₁₆ is selected from lysine, arginine and histidine;
AA₁₇ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₁₈ is selected from leucine, isoleucine, valine and methionine;
AA₁₉ is selected from lysine, arginine and histidine;
AA₂₀ is selected from serine, threonine, alanine, glycine and proline;
AA₂₁ is selected from lysine, arginine and histidine;
AA₂₂ is selected from serine, threonine, alanine, glycine and proline;
AA₂₃ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₂₄ is selected from serine, threonine, alanine, glycine and proline;
AA₂₅ is selected from lysine, arginine and histidine;
AA₂₆ is selected from lysine, arginine and histidine;
AA₂₇ is selected from leucine, isoleucine, valine and methionine;
AA₂₈ is selected from serine, threonine, alanine, glycine and proline;
AA₂₉ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₃₀ is selected from lysine, arginine and histidine;
AA₃₁ is selected from glutamine, glutamic acid, aspartic acid and asparagine;
AA₃₂ is selected from leucine, isoleucine, valine and methionine;
AA₃₃ is selected from leucine, isoleucine, valine and methionine;
AA_{34} is selected from serine, threonine, alanine, glycine and proline;
AA_{35} is selected from lysine, arginine and liistidine;
AA_{36} is selected from serine, threonine, alanine, glycine and proline;
AA_{37} is selected from leucine, isoleucine, valine and methionine;
AA_{38} is selected from tryptophan, phenylalanine and tyrosine;
AA_{39} is selected from tryptophan, phenylalanine and tyrosine;
AA_{40} is selected from serine, threonine, alanine, glycine and proline;
AA_{41} is selected from tryptophan, phenylalanine and tyrosine;
AA_{42} is selected from tryptophan, phenylalanine and tyrosine;
AA_{43} is selected from leucine, isoleucine, valine and methionine;
AA_{44} is selected from serine, threonine, alanine, glycine and proline;
AA_{45} is selected from serine, threonine, alanine, glycine and proline;
AA_{46} is selected from serine, threonine, alanine, glycine and proline;
AA_{47} is selected from leucine, isoleucine, valine and methionine;
AA_{48} is selected from tryptophan, phenylalanine and tyrosine;
AA_{49} is selected from glutamine, glutamic acid, aspartic acid and asparagaine;
AA_{50} is selected from lysine, arginine and histidine; and
AA_{51} is selected from lysine, arginine and histidine.

Included are also modified peptides based on the full TIOl peptide, TIOl peptide or partial TIOl peptide, including the following subsequences (amino acid numbering based on the TIOl peptide):

AA_{38}-AA_{39}-AA_{40}-AA_{41}-AA_{42}; wherein AA_{38} and AA_{39} are Class VI amino acids, preferably tryptophan; AA_{40} is a Class II amino acid, preferably threonine; and AA_{41} and AA_{42} are Class VI amino acids, preferably phenylalanine.

AA_{38}-AA_{39}-AA_{40}-AA_{41}-AA_{42}; wherein AA_{38} and AA_{39} are Class VI amino acids, preferably tryptophan; AA_{40} is a Class II amino acid, preferably threonine; AA_{41} and AA_{42} are Class VI amino acids, preferably phenylalanine; and AA_{43} is a Class V amino acid, preferably leucine.

Ala-Val-AAs_{8}AA_{38}-AA_{39}-AA_{40}-AA_{41}-AA_{42}; wherein AA_{38} and AA_{39} are Class VI amino acids, preferably tryptophan; AA_{40} is a Class II amino acid, preferably threonine; and AA_{41} and AA_{42} are Class VI amino acids, preferably phenylalanine.

Ala-Val-AAs_{8}AA_{38}-AA_{39}-AA_{40}-AA_{41}-AA_{42}; wherein AA_{38} and AA_{39} are Class VI amino acids, preferably tryptophan; AA_{40} is a Class II amino acid, preferably threonine; and AA_{41} and AA_{42} are Class VI amino acids, preferably phenylalanine.
threonine; AA41 and AA42 are Class VI amino acids, preferably phenylalanine; and
AA43 is a Class V amino acid, preferably leucine.

The peptides and polypeptides used in the method of the invention may be manufactured by any conventional process such as chemical synthesis and recombinant technology.

The term "treating or preventing" in the context of the present invention refers to the administering of a therapeutic amount of the polypeptide or composition of the present invention which is effective to ameliorate undesired symptoms associated with a disease, to prevent the manifestation of such symptoms before they occur, to slow down the progression of the disease, slow down the deterioration of symptoms, to enhance the onset of remission period, slow down the irreversible damage caused in the progressive chronic stage of the disease, to delay the onset of said progressive stage, to lessen the severity or cure the disease, to improve survival rate or more rapid recovery, to prevent the disease form occurring, or a combination of two or more of the above. In addition, the term "treatment" in the context used herein also refers to prevention of the disease from occurring. The treatment (also preventative treatment) regimen and specific composition to be administered will depend on the type of disease to be treated and may be determined by various considerations known to those skilled in the art of medicine, e.g. the physicians.

The "therapeutically effective amount" for purposes herein is determined by such considerations as may be known in the art. The amount must be effective to achieve the desired therapeutic effect which depends on the type and mode of treatment. As is clear to the artisan, the amount should be effective to obtain the improvement of survival rate, to obtain a more rapid recovery, to obtain the improvement or elimination of symptoms or any other indicators as are selected as appropriate measures by those skilled in the art. Where, for example, the active ingredient is administered to treat an autoimmune disease, an effective amount of the active ingredient may be an amount which reduces the symptoms of the disease or even cures the disease for a limited or extended period of time.

A therapeutically effective amount of TIOI is typically administered in a single daily dose, although at times a daily dose may be divided into several doses administered throughout the day or at times several daily doses may be combined into a
single dose to be given to the patient once every several days, particularly if administered in a sustained release formulation.

The active ingredient may be administered as a non-active substance (e.g., pro-drug) and be made active only upon further modification/s by a natural process at a specific site in the subject. In any case, the derivative will be such that the therapeutic functionality of the pharmaceutical composition of the invention is preserved. Such pro-drugs are also encompassed by the term "active ingredient" as used herein.

The method of the invention may also include the administration of drugs in addition to the isolated polypeptide. For example, glucocorticoid drugs may be administered before, together with or subsequently to the administration of the isolated polypeptide. Non-limiting examples of glucocorticoid drugs include dexamethasone, Cortisol, cortisone, prednisolone and prednisone.

A further embodiment of the invention relates to a pharmaceutical composition comprising an effective amount of an isolated polypeptide from the group consisting of:

(a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

(b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 15 in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1;

for use in a method for treating or preventing a disease capable of being alleviated by interleukin 10 (IL-10) and/or IL-4 in a subject in need.

The administration of the polypeptide to a patient may be together with a pharmaceutically acceptable carrier.

By the term "pharmaceutically acceptable carrier" it is meant any one of inert, non-toxic materials, which do not react with the active ingredient. The carrier is selected at times based on the desired form of the formulation. The carrier may also at times have the effect of the improving the delivery or penetration of the active ingredient to
the target tissue, for improving the stability of the drug, for slowing clearance rates, for imparting slow release properties, for reducing undesired side effects etc. The carrier may also be a substance that stabilizes the formulation (e.g. a preservative), for providing the formulation with an edible flavor, etc. The carriers may be any of those conventionally used and is limited only by chemical-physical considerations, such as solubility and lack of reactivity with the polypeptide, and by the route of administration. The carrier may include additives, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. In addition, the carrier may be an adjuvant, which, by definition are substances affecting the action of the active ingredient in a predictable way. Typical examples of carriers include (a) liquid solutions, where an effective amount of the active substance is dissolved in diluents, such as water, saline, natural juices, alcohols, syrups, etc.; (b) capsules (e.g. the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers), tablets, lozenges (wherein the active substance is in a flavor, such as sucrose and acacia or tragacanth or the active substance is in an inert base, such as gelatin and glycerin), and troches, each containing a predetermined amount of active agent as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; (e) suitable emulsions; (f) liposome formulation; and others.

When administering the compositions of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulation suitable for injection includes sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier employed can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, lipid polyethylene glycol and the like), suitable mixtures thereof and vegetable oils.

In any case, the pharmaceutical compositions of the invention are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient's age, sex, body weight and other factors known to medical practitioners.
The composition of the invention may be administered in various ways. It can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally or by intranasal administration, as well as by intrathecal and infusion techniques known to the man versed in the art. In a preferred embodiment, a pharmaceutical composition for oral administration comprises an isolated polypeptide in which one or more of the L-amino acids are replaced by the corresponding D-amino acids.

As is known, a treatment course in humans is usually longer than in animals, e.g. mice, as exemplified herein. The treatment has a length proportional to the length of the disease process and active agent effectiveness. The therapeutic regimen may involve single doses or multiple doses over a period of several days or more. The treatment generally has a length contingent with the course of the disease process, active agent effectiveness and the patient species being treated.

Non-aqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and ester, such as isopropyl myristate, may also at times be used as solvent systems for the active ingredient.

Additionally, various additives which enhance the stability, sterility and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents and buffers can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid and the like.

For the purpose of oral administration, the active ingredient may be formulated in the form of tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like, are usable and may be obtained by techniques well known to the pharmacists.

A still further embodiment of the invention relates to a use of an effective amount of an isolated polypeptide from the group consisting of:

(a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

(b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;
(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1;

in the preparation of a pharmaceutical composition for use in a method for treating or preventing a disease capable of being alleviated by interleukin 10 (IL-10) and/or IL-4 in a subject in need.

Another aspect of the invention relates to a method for treating a subject in need by modulating the immunological stimulating activity of dendritic cells (DC) of the subject, comprising:

(a) removing blood from the subject;

(b) isolating DC and/or monocytes from the blood;

(c) optionally, if monocytes are present, transforming them into DC;

(d) bringing said DC into contact with an isolated polypeptide from the group consisting of:

   i) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

   ii) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1 in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

   iii) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

   iv) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1, and

(e) returning the treated DC to the subject, thereby treating the subject.

Still another aspect of the invention relates to a method of inducing apoptosis in inflammatory cells and/or in cells involved in an autoimmune disease, comprising
administering to the subject a therapeutically effective amount of an isolated polypeptide from the group consisting of:

(a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

(b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1.

In this aspect of the invention, apoptosis is induced directly in the inflammatory cells by the polypeptide, and not necessarily through induction of IL-10 or IL-4. "Inflammatory cells" are cells involved in the pathology of inflammatory diseases such as sepsis, endotoxemia, pancreatitis, uveitis, hepatitis, peritonitis, keratitis, SIRS and injury-induced inflammation. "Cells involved in an autoimmune disease" are cells which contribute to the development or pathology of an autoimmune disease. A non-exclusive list of autoimmune diseases appears above.

The present invention is defined by the claims, the contents of which are to be read as included within the disclosure of the specification, and will now be described by way of example with reference to the accompanying Figures. It is to be understood, that the terminology which has been used is intended to be in the nature of words of description rather than limitation.

While the foregoing description describes in detail only a few specific embodiments of the invention, it will be understood by those skilled in the art that the invention is not limited thereto and that other variations in form and details may be possible without departing from the scope and spirit of the invention herein disclosed.
BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Figs. 1A, 1B, 1C, 1D and 1E show bar graphs which indicate the levels of various cytokines in human peripheral blood lymphocytes (PBLs) treated with 1 \( \mu \)g/ml TIOI peptide for 48 hours. The amounts are based on RNA array analysis of PCR gel bands. Fig. 1A shows the level of interferon-\( \gamma \) (INF-\( \gamma \)) in the absence (Control) or presence (TIOI) of TIOI. Fig. 1B shows the level of Interleukin 10 (IL-10), Fig. 1C shows the level of IL-I\( \alpha \) receptor, Fig. 1D shows the level of IL-4, and Fig. 1E shows the level of IL-I\( \alpha \).

Figs. 2A and 2B show bar graphs which indicate the levels of INF-\( \gamma \) and IL-10 in mouse splenocytes treated with either TIOI peptide (Fig. 2A) or 13aa TIOI peptide (Fig. 2B), or saline (Control);

Fig. 3 is a bar graph showing IL-10 levels in mice injected with TIOI in saline as compared to a control of saline injections alone (Con) every other day. One experimental group received TIOI peptide injection once every other day (1/2), a second group once daily (1/1) and a third group twice daily (2/1);

Fig. 4 is a bar graph showing the mean colon length of mice in which IBD was induced by receiving 5\% DSS through the drinking water. Three groups of mice were tested: one group received no DSS and no treatment (water); a second group received DSS in the drinking water and was treated with saline injection (DSS w saline); the third group received DSS in the drinking water and was treated with injections of saline that contained TIOI (DSS w TIOI);

Fig. 5 is a graph showing the change in weight (gr) over time (days) of the groups of mice described in Fig. 4;

Figs. 6A, 6B and 6C show bar graphs indicating the relative amounts of interleukin-specific cDNA obtained from RNA extracted from colon lymph nodes of the mice described in Fig. 4. Fig. 6A shows the levels of INF-\( \gamma \) RNA. Fig. 6B shows the levels of IL-4 RNA and Fig. 6C shows the levels of IL-10 RNA;

Figs. 7A, 7B, 7C and 7D show a FACS analysis of mouse splenocytes. The cells were detected for the presence of either FITC (FL1-H) or PE (FL2-H). Fig. 7A shows the analysis of unlabelled cells (control); Fig. 7B shows the analysis of cells labeled
with FITC-αTIOL; Fig. 7C shows the analysis of cells labeled with PE-anti-mouse CD11c; and Fig. 7D shows the analysis of cells labeled with both PE-anti-mouse CD11c and FITC-anti-TIOl;

**Fig. 8** shows a bar graph illustrating the effect of TIOl on the level of TGF-β in mice with IBD, as described in Fig. 4;

**Fig. 9** shows a bar graph showing the mean colon length of mice treated as described in Fig. 4. The darker (left-hand) bars show results from mice injected with TIOl/saline simultaneously with receiving the DSS, while the lighter (right-hand) bars show results from mice injected with TIOl/saline after signs of IBD appeared;

**Figs. 10A and 10B** shows pictures of histological sections of colon taken from the mice of Fig. 9 who received saline (A) or TIOl (B); and

**Figs. HA, HB and HC** are graphs showing the results of the treatment of CIA mice with saline (○) or TIOl (●). Fig. HA shows the change in the Arthritis Index as a function of days after immunization, Fig. HB shows the change in paw thickness (x 0.01mm) and Fig. HC shows the change in the number of arthritic paws.

**DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS**

**Materials and Methods**

TIOl and derivatives thereof were obtained from Anaspec, Inc. (California). Each peptide has greater than 95% purity.

Purification of total RNA from cells was carried out using the Promega SV RNA isolation kit.

Reverse transformation of mRNA to cDNA was carried out using the Biological Industries, Ltd. (Israel) kit - EZ single strand cDNA.

RT-PCR (using a GHM0055 kit from Biosource, Inc. (USA)) was carried out using specific oligos for the different cytokines.

A typical RT-PCR procedure was as follows: 3 microliters from each of the cDNA, 5 microliters of the specific oligos (total), 20 microliters of Readymix Taq polymerase and 17 microliters of DDW, were used.

The following PCR procedure was employed:

- 1 min at 95°C
- 30 cycles of:
  1 min at 95°C;
After finishing the cycles, samples were kept at 72°C for 10 min.

FACS analysis was carried out using a Becton Dickenson FACS analyzer.

Quantification of the PCR bands was carried out using a BioRad EQ Chemidoc device.

**EXAMPLES**

**Example 1**

The effect of human TlOl peptide (SEQ ID NO:1) on the production of cytokines by human peripheral blood lymphocytes (PBL) was investigated.

Human PBLs were fractionated from blood using a Ficoll-Hypaque gradient and incubated at a cell concentration of 10^6 cells/ml for 48 hours at 37°C with 1 µg/ml or no (control) TlOl in RPMI medium + 10% FCS. Total RNA was harvested and cDNA was prepared. The levels of IL-1α, IL-4, IL-10 and IL-1α receptor were measured by RT-PCR.

As can be seen in Figs. 1A to ID, the levels of IL-1α, IL-4, IL-10 and IL-1α receptor, IL-4 and IL-10 significantly increased as a result of incubation with TlOl, while the level of INF-γ slightly decreased. The increase ranged between a 2X increase (IL-4) to a 4.7X increase (IL-10). Thus it can be seen that the TlOl peptide specifically activates Th2 cells (which secrete IL-1α, IL-4 and IL-10) and does not affect or inhibits Th1 cells (which secrete INF-γ).

**Example 2**

In this example, the effect of human TlOl peptide on the production of cytokines by mouse spleenocytes was investigated.

Mouse spleenocytes at a cell concentration of 10^6 cells/ml were incubated for 24 hours with 0.1 µg/ml or no (control) TlOl peptide or human partial 13aa TlOl peptide (SEQ ID NO: 5) under the conditions of Example 1. Total RNA was harvested and cDNA was prepared. The levels of IL-10 and INF-γ were measured by RT-PCR.

As can be seen in Figs. 2A and 2B, the level of IL-10 increased significantly, while the level of INF-γ was unaffected or decreased. These results support the finding that the TlOl peptide specifically enhances IL-10 production. This further supports the
conclusion that the TlOl peptide specifically activates Th2 cells as opposed to Th1 cells. Furthermore, the results obtained with the 13aa peptide indicate that this partial TlOl peptide contains the active portion of the TlOl peptide.

Example 3

The level of IL-10 was assayed in three groups of 3 mice each: (1) one group of mice was injected with TlOl twice a day (2/1); (2) another group was injected with the same concentration of TlOl once a day (1/1); (3) the third group of mice was injected once every other day (1/2). All injected doses were the same (72 micrograms/Kg weight). Mice injected with saline once every other day served as control (Con). The results are shown in Fig. 3.

As shown, the level of IL-10 was increased in all groups following the injection of TlOl peptide to these mice. The best regimen of injections was twice a day.

Example 4

In order to test the ability of TlOl to treat autoimmune diseases, a mouse model for colitis/Crohn's disease was used.

Twenty nine-week-old male Balb/c mice (21-25 grams) received water or water containing dextran sulfate (DSS) (5%) for 9 days. DSS induces a Crohn's-like disease (IBD - inflammatory bowel disease) in mice. During that time the mice were injected every other day with either saline or saline containing TlOl. The body weight of the mice was followed. At the end of the experiment the mice were sacrificed, the colon removed and its length measured.

The mice were divided into 3 groups:

Group #1 (4 mice) - Control - received normal drinking water (with no DSS) and no treatment;

Group #2 (8 mice) - DSS w saline - received drinking water that contained 5% DSS and were injected with saline;

Group #3 (8 mice) - DSS w BTL1 - received drinking water that contained 5% DSS and were injected i.p. with TlOl (at a dose of 60 μg/Kg).

The results are summarized in Figs. 4 and 5. It can be seen that the treatment with TlOl peptide caused an increase in mean colon length and body weight as compared to the diseased mice who received saline. The increase was found to be
statistically significant (results not shown). All of the mice in Group #2 had an enlarged spleen, as opposed to only one in Group #3. Thus, TIOI had a profound effect in decreasing disease symptoms in these animals, as compared with the untreated mice.

5 Example 5

In order to determine the amount of interleukins produced in the diseased tissues of the mice of Example 4, RNA was extracted from the colon lymph nodes of the mice, and cDNA was prepared from the RNA by RT-PCR and was analyzed for the presence of DNA encoding INF-γ, IL-4 and IL-10. The results are shown in Fig. 6.

   In Fig. 6A it may be seen that the TIOI peptide had no effect on IFN-γ levels in the colon lymph nodes. In contrast, the level of IL-4 was significantly increased in the group of mice treated with TIOI peptide (Fig. 6B). The results are especially striking with respect to IL-10 as shown in Fig. 6C. It can be seen that the colon lymph nodes of the diseased mice (Group #2) had no IL-10 at all. However, after treatment with the TIOI peptide, the level of IL-10 increased to double the level in the control group.

   This example provides striking evidence that the TIOI peptide significantly increases the endogenous IL-10 level, thereby bringing about an alleviation of autoimmune disease symptoms.

20 Example 6

Dendritic cells (DC) are defined by their ability to activate and prime naïve resting T cells and initiate an immune response [5]. They are antigen binding cells (APC) which are often found at the site of autoimmune disease. Certain subsets of DC express the CD33 antigen, which can serve as a marker for these cells.

   In order to determine whether DC cells specifically bind TIOI peptide, $10^6$ mouse splenocytes/tube in 10% FCS were incubated with various combinations of PE-anti-mouse CDIIC, which binds to mouse DC, and FITC-anti-TIOI which binds TIOI receptor. The cells were analyzed by FACS analysis, and the results are presented in Figs. 7A to 7D. A quantitative analysis of the % of cells in each quadrant of the figures follows (UL = upper left; UR = upper right; LL = lower left; LR = lower right).

<table>
<thead>
<tr>
<th>Quad</th>
<th>Events</th>
<th>%</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
</table>

Fig. 7A
It may be seen from Fig. 7C, as compared to Fig. 7A, that a portion of the splenocytes displays the CDllc antigen, indicating the presence of DC. From Fig. 7B it
may be seen that some of the splenocytes have TlOl receptors. Fig. 7D shows that some of the cells which have a TlOl receptor are DC. This indicates that TlOl is involved in immune response initiated by DC.

Example 7

Th-3-type cells are a unique T-cell subset which primarily secrete the factor TGF-β, provide help for IgA and have suppressive properties for Th-1 and other immune cells. In order to ascertain the effect of TlOl on TGF-β, the level of TGF-β was determined in the tissue described in Example 5. The results are shown in Fig. 8.

It may be seen that TlOl stimulates the amount of TGF-β in the diseased tissue, indicating another mechanism of suppressing Th-1-mediated inflammatory response.

Example 8

The experiment described in Example 4, above, was repeated. The mice were divided into 2 groups. In one group (15 mice), the mice were injected three times a week with TlOl/saline simultaneously with receiving the DSS for eight days, while in the second group (13 mice), the mice were injected with TlOl/saline after signs of IBD appeared once every other day. The amount of TlOl injected was 50 µg/kg body-weight = 1 µg per mouse. At the end of the experiment the mice were sacrificed, the colons removed, and the colon lengths were measured and averaged. The results are presented in Fig. 9.

It may be seen that the treatment with TlOl peptide caused a statistically significant increase in mean colon length as compared to the diseased mice who received saline. It appears that there is no significant difference between treating the mice before or after appearance of the disease symptoms.

Histological sections of the colons were taken from diseased mice who were treated with saline or TlOl, and are shown in Fig. 10. In the diseased mice treated with saline (Fig. 10(A)), the colon structure is degraded and the colon is exsanguinated, while in the mice treated with TlOl (Fig. 10(B)), the colon shows a healthy structure and is provided with blood vessels.
Example 9

Collagen-induced arthritis (CIA) is a mouse model for human rheumatoid arthritis. In this example, the effect of T1Ol on CIA was investigated.

Induction of arthritis

Bovine type II collagen (Sigma) was dissolved in 0.05 M acetic acid to a concentration of 2 mg/ml by stirring overnight at 4°C and was emulsified in an equal volume of Complete Freund's Adjuvant (CFA). DBA1 mice were immunized intradermally at the base of the tail with 100 µg collagen. On day 21 after priming, the mice received an intradermal booster injection with 100 µg collagen in Incomplete Freund's Adjuvant (IFA). For the synchronous onset of arthritis, 40 µg LPS (Sigma, St. Louis, MO) were injected i.p. on day 28. T1Ol was injected ip into the mice 4 days after LPS injection.

Disease evaluation

Mice were monitored every other day using the following numbered macroscopic criteria ranging from 0 to 4:

0 = normal;
1 = detectable arthritis with erythma;
2 = significant swelling and redness;
3 = severe swelling and redness from joint to digit; and
4 = maximal swelling and deformity with ankylosis.

The average of macroscopic score was expressed as a cumulative value for all paws, with a maximum possible score of 16 per mouse. The thickness of each paw was also measured with a caliper. The paw swelling for each mouse was calculated by adding the thicknesses of all four paws. In addition, the numbers of arthritic paws per mouse were added to represent the number of arthritic paws in a group, with a maximum possible score of 80 per group of 20 mice. The results are presented in Fig. 11.

Results

Fig. HA shows the Arthritics Index. The macroscopic score (mean ± CD) was expressed as a cumulative value for all paws, with a maximal possibility score of 16 (n=20, p<0.0001). Fig. HB shows the degree of paw swelling. The thickness of each
paw was also evaluated with spring-loaded caliper. The paw's swelling for each mouse was calculated by adding the thickness of the individual paws (pO.OOO1). Fig. HC shows the number of arthritic paws. The arthritic paws of individual mice were counted and totaled to represent the number of arthritic paws in each experimental group (maximum 80, pO.OOO1).

All of the above types of measurements clearly show that TIO1 significantly improves the wellbeing of the CIA mice, and indicate that TIO1 can be an effective therapeutic agent for the treatment of rheumatoid arthritis and other autoimmune diseases.
CLAIMS:

1. A method for treating or preventing a disease capable of being alleviated by interleukin 10 (IL-10) and/or IL-4 in a subject in need, comprising administering to the subject a therapeutically effective amount of an isolated polypeptide from the group consisting of:
   (a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;
   (b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;
   (c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and
   (d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1.

2. The method according to claim 1 wherein the disease is one of the group consisting of infectious diseases, acute and chronic inflammatory diseases, cancer, transplantation rejection and autoimmune diseases.

3. The method according to claim 2 wherein the inflammatory disease is one of the group consisting of sepsis, endotoxemia, pancreatitis, uveitis, hepatitis, peritonitis, keratitis, SIRS and injury-induced inflammation.

4. The method according to claim 2 wherein the autoimmune disease is one of the group consisting of multiple sclerosis (MS), autoimmune uveitis, autoimmune uveoretinitis, autoimmune thyroiditis, Hashimoto's disease, insulitis, Sjogren's syndrome, spontaneous abortions, experimental autoimmune myocarditis, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Crohn's disease, lupus (SLE), psoriasis, diabetes, particularly type I, Acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Allergic asthma, Allergic rhinitis, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome (APS), Autoimmune aplastic anemia, Autoimmune dysautonomia, Autoimmune hepatitis, Autoimmune hyperlipidemia, Autoimmune...
immunodeficiency, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune thrombocytopenic purpura (ATP), Axonal & neuronal neuropathies, Bal’s disease, Behnet's disease, Bullous pemphigoid, Cardiomyopathy, Castleman disease, Celiac sprue (nontropical), Chagas' disease, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatomyositis, Devic disease, Discoid lupus, Dressier's syndrome, Endometriosis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evan's syndrome, Fibromyalgia fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia, Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, Immunoregulatory lipoproteins, Inclusion body myositis, Insulin-dependent diabetes (type I), Interstitial cystitis, Juvenile arthritis, Juvenile diabetes, Kawasaki syndrome, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lyme disease, Meniere's disease, Microscopic polyangiitis, Mixed connective tissue disease (MCTD), Moore's ulcer, Mucha-Habermann disease, Myasthenia gravis, Myositis, Narcolepsy, Neutropenia, Ocular cicatricial pemphigoid, Osteoarthritis, Palindromic rheumatism, Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis, Psoriatic arthritis, Idiopathic pulmonary fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynaud's phenomenon, Reflex sympathetic dystrophy, Reiter's syndrome, Relapsing polychondritis, Restless legs syndrome, Rheumatic fever, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis (SBE), Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis/Giant cell arteritis,
Tbrombocytopenic purpura (TTP), Autoimmune thyroid disease, Tolosa-Hunt syndrome, Transverse myelitis & necrotizing myelopathy, Ulcerative colitis, Undifferentiated connective tissue disease (UCTD), Vasculitis, Vesiculobullous dermatosis, Vitiligo and Wegener's granulomatosis.

5. The method according to claim 2 wherein the infectious disease is one of the group resulting from bacterial, fungal or protozoan infections.

6. A method according to any of claims 1 to 5 wherein said isolated peptide is administered to the subject together with a glucocorticoid drug.

7. The method of claim 6 wherein said glucocorticoid is dexamethasone, Cortisol, cortisone, prednisolone and/or prednisone.

8. Use of an effective amount of an isolated polypeptide from the group consisting of:
   (a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;
   (b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;
   (c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and
   (d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1,

in the preparation of a pharmaceutical composition for use in a method for treating or preventing a disease capable of being alleviated by IL-10 and/or IL-4 in a subject in need.

9. A pharmaceutical composition comprising an effective amount of an isolated polypeptide from the group consisting of:
   (a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;
   (b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or
replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1, and a pharmaceutically acceptable carrier,

for use in a method for treating or preventing a disease capable of being alleviated by IL-10 and/or IL-4 in a subject in need.

10. A pharmaceutical composition according to claim 9 for oral administration.

11. A pharmaceutical composition according to claim 10 wherein one or more of the L-amino acids of the isolated polypeptide are replaced by the corresponding D-amino acids.

12. A method of inducing apoptosis in inflammatory cells and/or cells involved in an autoimmune disease, comprising administering to the subject a therapeutically effective amount of an isolated polypeptide from the group consisting of:

(a) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

(b) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

(c) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

(d) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1.

13. A method for treating a subject in need by modulating the immunological stimulating activity of dendritic cells (DC) of the subject, comprising:

(a) removing blood from the subject;

(b) isolating DC and/or monocytes from the blood;

(c) optionally, if monocytes are present, transforming them into DC;
(d) bringing said DC into contact with an isolated polypeptide from the group consisting of:

i) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1;

ii) an isolated polypeptide comprising an amino acid sequence of SEQ. ID. NO: 1, in which one or more amino acid residues is added, deleted or replaced, without significantly affecting the biological characteristics of the modified molecule as compared to the unmodified molecule;

iii) an isolated polypeptide comprising a partial contiguous sequence from SEQ. ID. NO: 1 that includes at least 8 amino acid residues, which contiguous sequence is included as a contiguous sequence in said SEQ. ID. NO: 1; and

iv) an isolated polypeptide comprising a contiguous sequence of 13 amino acid residues beginning from the N-terminal of SEQ. ID. NO: 1, and

(e) returning the treated DC to the subject, thereby treating the subject.
FIG. 2A

FIG. 2B
FIG. 8
FIG. 9