Title: ISOLATED POLYPEPTIDES OF CD44 AND USES THEREOF

Abstract: Isolated polypeptides of CD44 are provided. Accordingly, there is provided an isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3. Also provided is an isolated end-capping modified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, wherein the modified polypeptide comprises an anti-inflammatory activity. Also provided are compositions of matter, fusion proteins and pharmaceutical compositions and their use in the treatment of inflammatory disease.
FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to an isolated polypeptide of CD44 and, more particularly, but not exclusively, to an isolated polypeptide of CD44vRA and its use in the treatment of inflammatory disease.

CD44 is a cell surface adhesion molecule involved in multiple cellular functions, including cell-cell and cell-matrix interactions, cell migration, programmed cell death (apoptosis), or, conversely, cell survival and proliferation.

CD44 is the major cell surface receptor for hyaluronic acid (HA) but it has also been shown to bind proteins such as coUagens, fibronectin, fibrinogen, laminin, mucosal vascular addressin and osteopontin. CD44 is essential for recruitment of circulating lymphocytes to the site of inflammation and marked accumulation of CD44, and sometimes hyaluronic acid, is detected in areas of intensive cell migration and cell proliferation, as in wound healing, tissue remodeling, inflammation, morphogenesis and carcinogenesis.

The genomic sequence of mouse and human CD44 includes 5 constant exons at the 5' terminus, and 5 constant exons at the 3' end. The mouse CD44 gene includes 10 variant exons in the middle of the molecule, designated Vi-Vio, resulting in a total of 20 exons. The human CD44 gene comprises only 9 of these 10 variant exons (V2-Vio) thus comprising a total of 19 exons. Differential V2-Vio alternative splicing generates many isoforms of CD44 that express various combinations of variant exons (designated exon Vx, x = 1-10), which are inserted in the membrane proximal domain and constitute the variable region of the molecule. These molecules are designated CD44 variants (CD44v). A few dozens isoforms of CD44 are known to date.

CD44s, which does not contain any variant exon, is the most ubiquitous form and is expressed by most cell types [Ponta, H., et al. Nat Rev Mol Cell Biol. 2003 Jan;4(1):33-45]. CD44 variant proteins, in which one or more of the 10 variant exons are included, are mostly reported in association with cancer, and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis [see e.g. Naor et al. Adv. Cancer Res.,71, 241-319,1997; and Naor et al. Critical Reviews in Clinical Laboratory Sciences. 39, 527-579, 2002].
Joint inflammatory cells of patients with rheumatoid arthritis (RA) display a sequence of alternatively spliced CD44 variant designated CD44vRA. Human CD44vRA contains the same sequence as that of keratinocytes CD44v3-vlO isoform with an addition of extra alanine in the splicing junction between variant exon 4 and variant exon 5, which does not interfere with the reading frame. Mice with collagen-induced arthritis (CIA) contain at the same site a similar sequence that also includes the alanine. The CD44vRA sequence is expressed on joint inflammatory synovial cells of RA patients and Psoriatic Arthritis (PA) patients, but neither on keratinocytes nor peripheral blood leukocytes (PBLs) of healthy donors. Furthermore, while joint inflammatory cells of RA patients express CD44vRA, PBLs from the same patients and synovial fluid cells from osteoarthritis patients hardly express this variant, demonstrating the exclusivity of this isoform. (Nedvetzki et al., J Clin Invest 111:1211-1220, 2003; Golan et al., J Autoimm 28:99-113, 2007).


SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

According to an aspect of some embodiments of the present invention there is provided an isolated end-capping modified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, wherein the modified polypeptide comprises an anti-inflammatory activity.
According to some embodiments of the invention, the end-capping comprises an N terminus end-capping.

According to some embodiments of the invention, the N terminus end-capping comprises an Acetyl.

According to some embodiments of the invention, the end-capping comprises a C terminus end-capping.

According to some embodiments of the invention, the C terminus end-capping comprises an Amide.

According to some embodiments of the invention, the polypeptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

According to some embodiments of the invention, the polypeptide is as set forth in SEQ ID NO: 1.

According to some embodiments of the invention, the end-capping modified polypeptide being selected from the group consisting to SEQ ID NOs: 4-6.

According to some embodiments of the invention there is provided a composition of matter comprising the isolated polypeptide and a non-proteinaceous moiety attached to the isolated polypeptide, wherein the isolated fusion polypeptide comprises an anti-inflammatory activity.

According to some embodiments of the invention there is provided an isolated fusion polypeptide comprising the isolate polypeptide having a C and/or N terminally attached amino acid sequence, wherein the C terminally amino acid sequence is a non-contiguous CD44vRA amino acid sequence with the isolated fusion polypeptide; and wherein the fusion polypeptide comprises an anti-inflammatory activity.

According to some embodiments of the invention, the attached is covalent attachment.

According to some embodiments of the invention, the anti-inflammatory activity is not dependent on vaccination or mucosal tolerance.

According to some embodiments of the invention, the isolated polypeptide or the composition of matter being capable of binding a protein selected from the group consisting of serum amyloid A, Transthyretin and apolipoprotein B.
According to some embodiments of the invention there is provided a pharmaceutical composition comprising as an active agent the isolated polypeptide or the composition of matter; and a pharmaceutically acceptable carrier or diluent.

According to some embodiments of the invention there is provided a method of treating an inflammatory disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated polypeptide, the composition of matter or the pharmaceutical composition, thereby treating the inflammatory disease in the subject.

According to some embodiments of the invention there is provided a use of the isolated polypeptide, the composition of matter or the pharmaceutical composition, for the manufacture of a medicament for the treatment of an inflammatory disease.

According to some embodiments of the invention, the administering comprises oral administering.

According to some embodiments of the invention, the composition is formulated for oral administration.

According to some embodiments of the invention, the inflammatory disease involves cells expressing CD44vRA.

According to some embodiments of the invention, the inflammatory disease is selected from the group consisting of Rheumatoid arthritis, psoriatic arthritis, Alzheimer's disease, cancer and cardiovascular disease.

According to some embodiments of the invention, the inflammatory disease is Rheumatoid arthritis.

According to some embodiments of the invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding the isolated polypeptide.

According to some embodiments of the invention there is provided a nucleic acid construct comprising the isolated polynucleotide.

According to some embodiments of the invention there is provided a method of determining potency of a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition, the method comprising:

(a) contacting a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition with fibroblasts obtained from an inflammatory joint of a Rheumatoid arthritis patient; and
(b) determining survival of the fibroblasts following a predetermined incubation time, so as to determine the potency of the batch.

According to some embodiments of the invention, the method comprising synthesizing the isolated polypeptide, the composition of matter or the pharmaceutical composition with a modification prior to the contacting.

According to some embodiments of the invention, reduced survival of the fibroblasts following the contacting is indicative that the batch is potent.

According to some embodiments of the invention, the method comprising comparing the survival of the cells with survival of the cells following contacting with a reference standard batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition, so as to determine the relative potency of the batch.

According to some embodiments of the invention, the method is effected *in-vitro* or *ex-vivo*.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

**BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS**

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 shows Liquid chromatography-mass spectrometry (LCMS) analysis demonstrating the % stability of the 5-mer RA peptide (SEQ ID NO: 1) following
storage at the indicated temperatures; assuming that storage at -20 °C represents 100% stability.

FIGS. 2A-C are graphs demonstrating that the 9-mer RA peptide (SEQ ID NO: 3) reduces joint inflammation in collagen-induced arthritis (CIA) mice on DBA/1 background. The Figures show paw swelling following injection of the 9-mer peptide at a dose of 25 µg (Figure 2A), 100 µg (Figure 2B) or 150 µg (Figure 2C) at the indicated time points (marked by arrow heads). PBS was injected as control. The y-axis represents Δ paw swelling indicating the difference (by mm) between the width of the paw at each of the measurement time points and the width of the paw at the onset of disease (time 0). The results are expressed as mean ± SE; the number of mice in each group (n) is indicated in insets of each Figure; * P < 0.05.

FIGS. 3A-C demonstrate that treatment with the 5-mer RA peptide (SEQ ID NO: 1) can restore normal histology of the inflamed joint in CIA mice on C57BL/6 background. Figures 3A and 3B are representative photomicrographs of H&E stained hind limb joint sections from mice treated with PBS control (Figure 3A) or 5-mer RA peptide (Figure 3B). Figure 3C is a graph summarizing the average inflammatory score as evaluated by histological examination of H&E stained hind limb joint sections from mice treated with PBS control (n = 7) or 5-mer RA peptide (n = 7), wherein 0 indicates no infiltration and 4 indicates massive infiltration. p < 0.0001.

FIG. 4 is a graph demonstrating that the 7- and 9-mer protected RA peptides (SEQ ID NOs: 5-6) reduce joint inflammation in CIA mice on DBA/1 background. The Figure shows paw swelling following injection of the peptides at a dose of 200 µg at the indicated time points (marked by arrows). PBS was injected as control. The y-axis represents Δ paw swelling indicating the difference (by mm) between the width of the paw at each of the measurement time points and the width of the paw at the onset of disease (time 0). The results are expressed as mean ± SE; the number of mice in each group (n) is indicated in insets of each Figure; * P < 0.05, ** p < 0.01.

FIGS. 5A-B are graphs demonstrating that the 5-mer protected RA peptide (SEQ ID NO: 4) reduces joint inflammation in CIA mice on DBA/1 background. The Figures show paw swelling following injection of the peptide at a dose of 200 µg at the indicated time points (marked by arrows). PBS (Figures 5A and 5B) or Dexamethasone (Dex) (Figure 5A) were injected as control. The y-axis represents Δ paw swelling
indicating the difference (by mm) between the width of the paw at each of the measurement time points and the width of the paw at the onset of disease (time 0). The results are expressed as mean ± SE; the number of mice in each group (n) is indicated in insets of each Figure; * P < 0.05, ** p < 0.01.

FIG. 6 is a graph demonstrating that the 5-mer protected RA peptide (SEQ ID NO: 4) reduces joint inflammation in CIA mice on C57BL background. The Figures show paw swelling following injection of the peptide at a dose of 70 µg for 10 consecutive days following onset of disease. PBS was injected as control. The y-axis represents Δ paw swelling indicating the difference (by mm) between the width of the paw at each of the measurement time points and the width of the paw at the onset of disease (time 0). The results are expressed as mean ± SE; * P < 0.006.

FIGs. 7A-C are graphs demonstrating that the 7-mer protected RA peptide, which includes the core MTADV sequence (SEQ ID NO: 5), reduces joint inflammation in CIA mice on DBA/1 background, while the non-specific core scrambled 7-mer peptide (SEQ ID NO: 7) has no effect on joint inflammation in this model. The Figures show paw swelling following injection of the peptides at a dose of 200 µg at the indicated time points (marked by arrows). PBS was injected as control. The y-axis represents paw swelling in mm (Figures 7A and 7C) or Δ paw swelling (Figure 7B) indicating the difference between the width of the paw at each of the measurement time points and the width of the paw at the onset of disease (time 0, Figure 7B). The results are expressed as mean ± SE; the number of mice in each group (n) is indicated in insets of each Figure; * P < 0.05, ** p < 0.005.

FIG. 8 is a bar graph showing the percentages of healthy hind paws in CIA mice following injection of 7-mer protected RA peptide (SEQ ID NO: 5) or non-specific scrambled 7-mer peptide (SEQ ID NO: 7) according to the experimental method described in Table 6. PBS was injected as control.

FIGs. 9A-B are graphs demonstrating that a dose of 70 µg per injection is the optimal dose for inhibiting joint inflammation in CIA mice on C57BL/6 background by the 5-mer protected RA peptide (SEQ ID NO: 4). The Figures show paw swelling following injection of the peptide at a dose of 70, 200 and 600 µg (Figure 9A) or 10, 25 and 70 µg (Figure 9B) for 10 consecutive days following onset of disease. PBS was injected as control. The y-axis represents Δ paw swelling indicating the difference
between the width of the paw at each of the measurement time points and the width of
the paw at the onset of disease (time 0). The results are expressed as mean ± SE; the
number of mice in each group (n) is indicated in insets of each graph; * P is indicated
on each graph.

FIG. 10 shows graphs demonstrating the effect of the 5-mer RA peptide (SEQ
ID NO: 1) on delayed type hypersensitivity (DTH) response in C57BL/6 mice. The y-
axis represents the difference in thickness between the right and the left ears on day 7.
Treatment with PBS and anti-TNFα served as positive and negative control, respectively. The results are expressed as mean ± SE. The DTH protocol comprised
sensitization with Oxazolone on day 0; elicitation (challenge) in the ear with Oxazolone
on day 6; and measurement of ear thickness day 7. PBS or peptide were injected from
day -1 to day 7.

FIG. 11 is a graph showing absence of neutralizing anti-peptide specific
antibodies in the serum of mice treated with the 5-mer peptide (SEQ ID NO: 1), as
determined by ELISA. ELISA plates coated with the 5-mer peptide or with collagen
and mouse IgG, which served as positive controls. Sera from mice treated with the 5-
mer peptide or with PBS were added to plate wells. Serum from naïve mice and mice
treated with PBS served as negative controls.

FIG. 12 is a schematic representation of the procedure used for identification
of the 5-mer peptide target proteins.

FIG. 13 is a graph showing the pharmacokinetic elimination of the 5-mer RA
peptide (SEQ ID NO: 1) in the serum of mice following a single injection of the
peptide.

FIG. 14 is a graph demonstrating the in vitro effect of the 5-mer RA peptide
(SEQ ID NO: 1) on survival of fibroblasts isolated from the inflammatory joint of an
RA patient, as determined by a MTT assay.

FIG. 15 is a graph demonstrating that Serum Amyloid A (SAA) prevents the in
vitro effect of the 5-mer RA peptide (SEQ ID NO: 1) on survival of fibroblasts isolated
from the inflammatory joint of an RA patient, as determined by a MTT assay. Lactalbumin (LA) was used as a non-specific control. The 5-mer peptide was added in
a constant concentration (25µg/ml); the x-axis indicates SAA and LA concentration.
FIG. 16 is a graph demonstrating the in vitro effect of the 5-mer RA peptide (SEQ ID NO: 1) on survival of fibroblasts isolated from the inflammatory joint of an RA patient in comparison to the 5-mer protected RA peptide (SEQ ID NO: 4), as determined by a MTT assay.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to an isolated polypeptide of CD44 and, more particularly, but not exclusively, to an isolated polypeptide of CD44vRA and its use in the treatment of inflammatory disease.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

CD44 is a cell surface adhesion molecule involved in multiple cellular functions, including cell-cell and cell-matrix interactions, cell migration, programmed cell death or, conversely, cell survival and proliferation. The genomic sequence of human CD44 includes 5 constant exons at the 5’ terminus and 5 constant exons at the 3’ terminus, as well as 9 variant exons encompassed therebetween. Several dozens of splice variants of CD44 are known to date. CD44s (SEQ ID NO: 9), which does not contain any variant exon, is the most ubiquitous form and is expressed by most cell types. Joint inflammatory cells of patients with psoriatic arthritis (PA), rheumatoid arthritis (RA) present a sequence of alternatively spliced CD44 variant designated CD44vRA (SEQ ID NO: 11), not expressed on keratinocytes nor peripheral blood leukocytes (PBLs) of healthy donors.

Whilst reducing the present invention to practice, the present inventors have now uncovered that peptides as short as 5, 7 or 9 mers comprising a MTADV sequence resulting from inclusion of alanine in the splicing junction between variant exon 4 and variant exon 5 of CD44vRA are capable of inhibiting joint inflammation in a CIA mouse model (the mouse analogue of human RA). Without wishing to be bound by theory it is believed that the polypeptides of some embodiments of the invention elicit their activity by competing with the natural ligand of CD44vRA.
As is illustrated hereinunder and in the examples section, which follows, the present inventors have synthesized 5-, 7- and 9-mer peptides (SEQ ID NOs: 1-3, also denoted herein as "RA peptides") and respective peptides with Acetyl and Amide protecting residues, at the amino and carboxyl terminal ends of the peptides respectively (SEQ ID NOs: 4-6, also denoted herein as "RA protected peptides"). The peptides comprise hydrophobic amino acids, no proteolytic sites and are stable at room temperature and 4 °C for at least 22 weeks (Example 1, Figure 1). The synthesized RA peptides and RA protected peptides were able to reduce joint inflammation in vivo in a CIA mouse model (Examples 2-4, Figures 2A-B, 3A-C; 4, 5-A-B, 6, 8 and 9A-B). Moreover, the peptides did not elicit generation of neutralizing anti-peptide specific antibodies nor affected general immune response as evaluated by delayed hypersensitivity (DTH) response (Example 5-6, Figures 10-11). Importantly, a scrambled non-specific 7-mer protected peptide (SEQ ID NO: 7) had no effect on joint inflammation in the CIA mouse model (Example 3 Figures 7A-B and 8). Mass spectrometry analysis further revealed few potential target proteins of the RA peptides, namely Serum amyloid A, Transthyretin and Apolipoprotein B (Example 7, Figure 12). In addition the inventors have developed a novel in-vitro assay to test the activity of the RA peptides and RA protected peptides (Example 9, Figures 14-16).

Consequently, the present teachings suggest the use of compositions comprising the RA- and RA-protected peptides in the treatment of inflammatory diseases.

Thus, according to a first aspect of the present invention, there is provided an isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

According to specific embodiments the polypeptide is as set forth in SEQ ID NO: 1.

According to specific embodiments the polypeptide is as set forth in SEQ ID NO: 2.

According to specific embodiments the polypeptide is as set forth in SEQ ID NO: 3.

According to an aspect of the present invention, there is provided an isolated end-capping modified polypeptide comprising an amino acid sequence selected from
the group consisting of SEQ ID NO: 1-3, wherein said modified polypeptide comprises an anti-inflammatory activity.

According to specific embodiments, the polypeptide amino acid sequence of the end-capping modified polypeptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

According to another aspect of the present invention there is provided a composition of matter comprising the isolated polypeptide and a non-proteinaceous moiety attached to the isolated polypeptide, wherein the isolated fusion polypeptide comprises an anti-inflammatory activity.

According to another aspect of the present invention there is provided an isolated fusion polypeptide comprising the isolated polypeptide having a C and/or N terminally attached amino acid sequence, wherein said C terminally amino acid sequence is a non-contiguous CD44vRA amino acid sequence with said isolated fusion polypeptide; and wherein said fusion polypeptide comprises an anti-inflammatory activity.

The terms "peptide" and "polypeptide" which are interchangeably used herein encompass native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body, more capable of penetrating into cells improving clearance, biodistribution and/or pharmacokinetics. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereunder.

Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated amide bonds (-N(CH3)-CO-), ester bonds (-C(=0)-O-), ketomethylene bonds (-CO-CH2-), sulfinylmethylene bonds (-S(=0)-CH2-), a-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl (e.g., methyl), amine bonds (-CH2-NH-), sulfide bonds (-CH2-S-), ethylene bonds (-CH2-CH2-), hydroxyethylene bonds (-CH(OH)-CH2-),
thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), fluorinated olefinic double bonds (-CF=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally present on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (e.g. 2-3) bonds at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted by non-natural aromatic amino acids such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), naphthylalanine, ring-methylated derivatives of Phe, halogenated derivatives of Phe or O-methyl-Tyr.

The peptides of some embodiments of the invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

The term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphoethreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylsine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids (stereoisomers).

Tables 1 and 2 below list naturally occurring amino acids (Table 1), and non-conventional or modified amino acids (e.g., synthetic, Table 2) which can be used with some embodiments of the invention.

Table 1

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Three-Letter Abbreviation</th>
<th>One-letter Symbol</th>
</tr>
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<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
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<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
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<td>N</td>
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<tr>
<td>Aspartic acid</td>
<td>Asp</td>
<td>D</td>
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<td>Isoleucine</td>
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<td>Non-conventional amino acid</td>
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<td>2-aminoadipic acid</td>
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The amino acids of the polypeptides of the present invention may be substituted either conservatively or non-conservatively.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino or a peptidomimetics having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily determined bearing in mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered as conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acid is well documented in the literature known to the skilled practitioner.

When affecting conservative substitutions the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cyclohexylmethyl glycine for alanine, isoleucine for glycine, or -NH-CH\((\cdot\text{CH}_2)\text{S-COOH}\)-CO- for aspartic acid. Those non-conservative substitutions which fall under the scope of the present invention are those which still constitute a peptide having neuroprotective properties.
The peptides of some embodiments of the invention are preferably utilized in a linear form, although it will be appreciated that in cases where cyclicization does not severely interfere with peptide characteristics, cyclic forms of the peptide can also be utilized.

Since the present peptides are preferably utilized in therapeutics which requires the peptides to be in soluble form, the peptides of some embodiments of the invention preferably include one or more non-natural or natural polar amino acids, including but not limited to serine and threonine which are capable of increasing peptide solubility due to their hydroxyl-containing side chain.

As mentioned, the N and C termini of the peptides of the present invention may be protected by functional groups. Suitable functional groups are described in Green and Wuts, "Protecting Groups in Organic Synthesis", John Wiley and Sons, Chapters 5 and 7, 1991, the teachings of which are incorporated herein by reference. Thus, the polypeptide may be modified at the N- (amine) terminus and/or the C- (carboxyl) terminus thereof so as to produce an end capping modified peptide.

As used herein, the phrases "end-capping modified polypeptide" and "protected polypeptide", which are interchangeably used herein, refer to a polypeptide which has been modified at the N- (amine) terminus and/or the C- (carboxyl) terminus thereof. The end-capping modification refers to the attachment of a chemical moiety to the terminus of the polypeptide, so as to form a cap. Such a chemical moiety is referred to herein as an end capping moiety and is typically also referred to herein and in the art, interchangeably, as a peptide protecting moiety or group. Hydroxyl protecting groups include but are not limited to esters, carbonates and carbamate protecting groups. Amine protecting groups include but are not limited to alkoxy and aryloxy carbonyl groups. Carboxylic acid protecting groups include but are not limited to aliphatic, benzylic and aryl esters.

The phrase "end-capping moiety", as used herein, refers to a moiety that when attached to the terminus of the peptide, modifies the N and/or C terminal ends(s) of the peptide. The end-capping modification typically results in masking the charge of the peptide terminus, and/or altering chemical features thereof, such as, hydrophobicity, hydrophilicity, reactivity, solubility and the like. By selecting the nature of the end capping modification, the hydrophobicity/hydrophilicity, as well as the solubility of the
peptide can be finely controlled. According to specific embodiments, the protecting groups facilitate transport of the peptide attached thereto into a cell. These moieties can be cleaved \textit{in vivo}, either by hydrolysis or enzymatically, inside the cell.

According to specific embodiments, the end-capping modification does not compromise the biological activity (i.e. anti-inflammatory activity) of the polypeptide. Examples of moieties suitable for peptide end-capping modification can be found, for example, in Green \textit{et al.}, "Protective Groups in Organic Chemistry", (Wiley, 2.sup.nd ed. 1991) and Harrison \textit{et al.}, "Compendium of Synthetic Organic Methods", Vols. 1-8 (John Wiley and Sons, 1971-1996).

According to specific embodiments, the end-capping comprises an N terminus end-capping.

Representative examples of N-terminus end-capping moieties include, but are not limited to, formyl, acetyl (also denoted herein as "Ac"), trifluoroacetyl, benzyl, benzyloxy carbonyl (also denoted herein as "Cbz"), tert-butoxy carbonyl (also denoted herein as "Boc"), trimethylsilyl (also denoted "TMS"), 2-trimethylsilyl-ethanesulfonyl (also denoted "SES"), trityl and substituted trityl groups, allyloxy carbonyl, 9-fluorenymethyloxycarbonyl (also denoted herein as "Fmoc"), and nitroveratryloxy carbonyl ("NVOC").

According to specific embodiments, the N terminus end-capping comprises an Acetyl.

According to specific embodiments, the end-capping comprises a C terminus end-capping.

Representative examples of C-terminus end-capping moieties are typically moieties that lead to acylation of the carboxy group at the C-terminus and include, but are not limited to, benzyl and trityl ethers as well as alkyl ethers, tetrahydropyranyl ethers, trialkysilyl ethers, alky1 ethers, monomethoxy trityl and dimethoxy trityl. Alternatively the \textit{-COOH} group of the C-terminus end-capping may be modified to an amide group.

According to specific embodiments, the C terminus end-capping comprises an Amide.
Other end-capping modifications of peptides include replacement of the amine and/or carboxyl with a different moiety, such as hydroxyl, thiol, halide, alkyl, aryl, alkoxy, aryloxy and the like.

According to specific embodiments, the peptide is modified only at the N-terminus or the C-terminus thereof.

According to other specific embodiments, the peptide is modified at both the N-terminus and the C-terminus.

According to specific embodiments, the peptide is modified at the N-terminus with an Acetyl and at the C terminus with an Amide.

According to specific embodiments the end-capping modified polypeptide is selected form the group consisting to SEQ ID NOs: 4-6.

The present invention further provides polypeptide conjugates and fusion polypeptides comprising peptides, analogs and derivatives according to the invention.

Thus, as mentioned, according to an aspect of the present invention there is provided an isolated fusion polypeptide comprising the isolated polypeptide having a C and/or N terminally attached amino acid sequence, wherein said C terminally amino acid sequence is a non-contiguous CD44vRA amino acid sequence with said isolated fusion polypeptide; and wherein said fusion polypeptide comprises an anti-inflammatory activity.

As used herein, the phrase "non-contiguous CD44vRA amino acid sequence" refers to a fusion polypeptide that does not comprise an amino acid sequence of SEQ ID NOs: 1, 2 or 3 directly attached in its C terminus to an amino acid sequence of CD44vRA starting at coordinates 306, 308 or 308, respectively, of SEQ ID NO: 11.

According to specific embodiments, the isolated polypeptide and the attached amino acid sequence are covalently attached, directly or through a spacer or a linker which can be a synthetic or an amino acid linker.

As used herein the term "CD44" refers to the cell surface protein that is expressed in a large number of mammalian cell types and is encoded by the CD44 gene. According to a specific embodiment the CD44 is the human CD44 gene. The standard isoform, designated CD44, comprising exons 1-5 and 16-20 is expressed in most cell types and is set forth in GeneBank Accession Numbers NM_000610 and NP_000601 (SEQ ID NOs: 8 and 9).
As used herein the term "CD44vRA" (SEQ ID NO: 10 and 11) refers to a CD44 variant which is expressed in inflammation sites, e.g. on synovial fluid cells of RA patients but not on PBLs of healthy individuals. CD44vRA variant is a naturally occurring sequence which is presumably produced by alternative splicing of the primary transcript of the known CD44 gene which occurs in cells in inflammatory sites (e.g. in joints of RA patients) and does not arise from truncation or mutation of the known CD44 gene. This CD44vRA variant sequence comprises Exons 1-5, 15-17 and 19 of the constant part of the CD44 gene as well as Exons 7-14 (v3-vl0) of the variable region of the gene. The variant coding sequence comprises three additional bases (CAG) that are transcribed from the end of the intron bridging Exon v4 to Exon v5 and are inserted at the 5' end of Exon v5. This extra CAG sequence results in the insertion of a new codon for the amino acid alanine in position 303 of SEQ ID NO: 11 while leaving the reading frame intact.

The terms "CD44" and "CD44vRA", also refer to CD44 and CD44vRA homologues which exhibit the desired activity (e.g. cell migration and/or cell-cell and cell-matrix interactions). Such homologues can be, for example, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide of SEQ ID NOs: 9 and 11, or 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same.

The homolog may also refer to an ortholog, a deletion, insertion, or substitution variant, including an amino acid substitution, as long as it retains the activity.

Sequence identity or homology can be determined using any protein or nucleic acid sequence alignment algorithm such as Blast, ClustalW, and MUSCLE.

According to other specific embodiments of the invention, the peptide is attached to a non-proteinaceous moiety.
According to specific embodiments, the isolated polypeptide and the attached non-proteinaceous moiety are covalently attached, directly or through a spacer or a linker.

The phrase "non-proteinaceous moiety" as used herein refers to a molecule not including peptide bonded amino acids that is attached to the above-described peptide. According to a specific embodiment the non-proteinaceous is a non-toxic moiety. Exemplary non-proteinaceous moieties which may be used according to the present teachings include, but are not limited to a drug, a chemical, a small molecule, a polynucleotide, a detectable moiety, polyethylene glycol (PEG), Polyvinyl pyrrolidone (PVP), poly(styrene comaleic anhydride) (SMA), and divinyl ether and maleic anhydride copolymer (DIVEMA). According to specific embodiments of the invention, the non-proteinaceous moiety comprises polyethylene glycol (PEG).

Such a molecule is highly stable (resistant to in-vivo proteolytic activity probably due to steric hindrance conferred by the non-proteinaceous moiety) and may be produced using common solid phase synthesis methods which are inexpensive and highly efficient, as further described hereinbelow. However, it will be appreciated that recombinant techniques may still be used, whereby the recombinant peptide product is subjected to in-vitro modification (e.g., PEGylation as further described hereinbelow).

Bioconjugation of the polypeptide amino acid sequence with PEG (i.e., PEGylation) can be effected using PEG derivatives such as N-hydroxysuccinimide (NHS) esters of PEG carboxylic acids, monomethoxyPEG(2)-NHS, succinimidyl ester of carboxymethylated PEG (SCM-PEG), benzotriazole carbonate derivatives of PEG, glycidyl ethers of PEG, PEG p-nitrophenyl carbonates (PEG-NPC, such as methoxy PEG-NPC), PEG aldehydes, PEG-orthopyridyl-disulfide, carbonylimidazol-activated PEGs, PEG-thiol, PEG-maleimide. Such PEG derivatives are commercially available at various molecular weights [See, e.g., Catalog, Polyethylene Glycol and Derivatives, 2000 (Shearwater Polymers, Inc., Huntsville, Ala.)]. If desired, many of the above derivatives are available in a monofunctional monomethoxyPEG (mPEG) form. In general, the PEG added to the polypeptide of the present invention should range from a molecular weight (MW) of several hundred Daltons to about 100 kDa (e.g., between 3-30 kDa). Larger MW PEG may be used, but may result in some loss of yield of PEGylated polypeptides. The purity of larger PEG molecules should be also watched,
as it may be difficult to obtain larger MW PEG of purity as high as that obtainable for lower MW PEG. It is preferable to use PEG of at least 85% purity, and more preferably of at least 90% purity, 95% purity, or higher. PEGylation of molecules is further discussed in, e.g., Hermanson, Bioconjugate Techniques, Academic Press San Diego, Calif. (1996), at Chapter 15 and in Zalipsky et al., "Succinimidyl Carbonates of Polyethylene Glycol," in Dunn and Ottenbrite, eds., Polymeric Drugs and Drug Delivery Systems, American Chemical Society, Washington, D.C. (1991).

Conveniently, PEG can be attached to a chosen position in the polypeptide by site-specific mutagenesis as long as the activity of the conjugate is retained. A target for PEGylation could be any Cysteine residue at the N-terminus or the C-terminus of the peptide sequence. Additionally or alternatively, other Cysteine residues can be added to the polypeptide amino acid sequence (e.g., at the N-terminus or the C-terminus) to thereby serve as a target for PEGylation. Computational analysis may be effected to select a preferred position for mutagenesis without compromising the activity.

Various conjugation chemistries of activated PEG such as PEG-maleimide, PEG-vinylsulfone (VS), PEG-acrylate (AC), PEG-orthopyridyl disulfide can be employed. Methods of preparing activated PEG molecules are known in the arts. For example, PEG-VS can be prepared under argon by reacting a dichloromethane (DCM) solution of the PEG-OH with NaH and then with di-vinylsulfone (molar ratios: OH 1: NaH 5: divinyl sulfone 50, at 0.2 gram PEG/mL DCM). PEG-AC is made under argon by reacting a DCM solution of the PEG-OH with acryloyl chloride and triethylamine (molar ratios: OH 1: acryloyl chloride 1.5: triethylamine 2, at 0.2 gram PEG/mL DCM). Such chemical groups can be attached to linearized, 2-arm, 4-arm, or 8-arm PEG molecules.

Resultant conjugated molecules (e.g., PEGylated or PVP-conjugated polypeptide) are separated, purified and qualified using e.g., high-performance liquid chromatography (HPLC) as well as biological assays.

According to specific embodiments, the CD44vRA peptide portion of the polypeptides of the invention other than those listed as consisting of SEQ ID NOs: 1-3 are 5-100, 5-50, or 5-40, or 5-20, 5-15, 5-10, 5-9, 5-7 amino acids in length.
According to specific embodiments, the peptide portion of the polypeptides of the invention does not comprise a CD44vRA amino acid sequence other than those listed as consisting of SEQ ID NOs: 1-3.

The peptides and compositions of matter of the present invention may be attached (either covalently or non-covalently) to a penetrating agent.

As used herein the phrase "penetrating agent" refers to an agent which enhances translocation of any of the attached peptide or composition of matter across a cell membrane.

According to one embodiment, the penetrating agent is a peptide and is attached to the polypeptide (either directly or non-directly) via a peptide bond.

Typically, peptide penetrating agents have an amino acid composition containing either a high relative abundance of positively charged amino acids such as lysine or arginine, or have sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids.

According to specific embodiments, the polypeptide is provided in a formulation suitable for cell penetration that enhances intracellular delivery of the polypeptide as further described hereinbelow.

By way of non-limiting example, cell penetrating peptide (CPP) sequences may be used in order to enhance intracellular penetration; however, the disclosure is not so limited, and any suitable penetrating agent may be used, as known by those of skill in the art.

Cell-Penetrating Peptides (CPPs) are short peptides (<40 amino acids), with the ability to gain access to the interior of almost any cell. They are highly cationic and usually rich in arginine and lysine amino acids. They have the exceptional property of carrying into the cells a wide variety of covalently and noncovalently conjugated cargoes such as proteins, oligonucleotides, and even 200 nm liposomes. Therefore, according to additional exemplary embodiment CPPs can be used to transport the polypeptide or the composition of matter to the interior of cells.

TAT (transcription activator from HIV-1), pAntp (also named penetratin, Drosophila antennapedia homeodomain transcription factor) and VP22 (from Herpes Simplex virus) are examples of CPPs that can enter cells in a non-toxic and efficient manner and may be suitable for use with some embodiments of the invention. Protocols for producing CPPs-cargos conjugates and for infecting cells with such conjugates can

The polypeptides of some embodiments of the invention may be synthesized by any techniques that are known to those skilled in the art of peptide synthesis, including solid phase and recombinant techniques.

Any of the proteinaceous polypeptides described herein can be encoded from a polynucleotide. These polynucleotides can be used as therapeutics per se or in the recombinant production of the agent.

Thus, according to an aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding the polypeptide of the present invention.

Thus, according to an aspect of the present invention there is provided a nucleic acid construct comprising the isolated polynucleotide.

Such a nucleic acid construct or system includes at least one cis-acting regulatory element for directing expression of the nucleic acid sequence. Cis-acting regulatory sequences include those that direct constitutive expression of a nucleotide sequence as well as those that direct inducible expression of the nucleotide sequence only under certain conditions. Thus, for example, a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner is included in the nucleic acid construct.

The isolated polypeptides and the compositions of matter of the present invention are endowed with anti-inflammatory activity.

As used herein, the phrase "anti-inflammatory activity" refers to prevention and/or reduction of acute and/or chronic inflammatory responses and/or in preventing and/or treating an inflammatory-related disease. Assays for qualifying an anti-inflammatory activity include but are not limited to those described in the Examples section which follows using both in-vitro and in-vivo models for inflammatory conditions (e.g. RA). Non-limiting examples include paw swelling in vivo in a CIA mouse model (see e.g. Nedvetzki, et al., (2004) PNAS 101, 18081-18086), histological examination of joint sections obtained from CIA mice and in-vitro cell viability of
fibroblasts obtained from synovial fluid of an RA patient as further described hereinbelow.

According to specific embodiments, the anti-inflammatory activity is not dependent on vaccination or mucosal tolerance.

According to specific embodiments, the isolated polypeptide of the composition of matter does not effect immune response in general, as may be evaluated by a delayed type hypersensitivity assay (DTH) such as disclosed in Weiss et al., (2000) Proc. Natl. Acad. Sci. USA. 97, 285-290; and in Example 5 in the Examples section which follows.

According to specific embodiments, the isolated polypeptide or the composition of matter is capable of binding a protein selected from the group consisting of serum amyloid A, Transthyretin and apolipoprotein B.

As used herein, the term "serum amyloid A" or "SAA" refer to the polynucleotide and expression product e.g., polypeptide of the SAA1, SAA2 and SAA4 genes. SAA1 is also known as serum amyloid Al, MGC1 11216, PIG4, SAA, and tumor protein p53 inducible protein 4 (TP53I4). According to specific embodiments the SAA1 refers to the human SAA1, such as provided in the following GeneBank Numbers NM_199161 and NM_000331 and Uniprot Number: P0DJI8 (SEQ ID NOs: 12-14). According to specific embodiments the SAA1 refers to the mouse SAA1, such as provided in the following GeneBank Number NM_00917 (SEQ ID NO: 15). SAA2 is also known as serum amyloid A2 and SAA. According to specific embodiments the SAA2 refers to the human SAA2, such as provided in the following GeneBank Numbers NM_001127380 and NM_030754 and Uniprot number P0DJI8 (SEQ ID NOs: 16-18). According to specific embodiments the SAA2 refers to the mouse SAA2, such as provided in the following GeneBank Numbers NM_011314 (SEQ ID NO: 19). According to specific embodiments the SAA4 refers to the human SAA4, such as provided in the following GeneBank Number NM_006512 and Uniprot Number P35542 (SEQ ID NOs: 20-21).

According to specific embodiments, the term "SAA" refers to SAA1 and SAA2 genes which belong to the serum amyloid A acute phase family of proteins.

As used herein, the term "Transthyretin", refers to the polynucleotide and expression product e.g., polypeptide of the TTR gene, which is a protein carrier of the
thyroid hormone thyroxine and retinol. According to specific embodiments the transthyretin refers to the human transthyretin, such as provided in the following GeneBank Numbers NP_000362 and NM_000371 (SEQ ID NOs: 22-23). According to other specific embodiments, the transthyretin refers to the mouse transthyretin, such as provided in the following GeneBank Numbers NP_038725 and NM_013697 (SEQ ID NOs: 24-25).

As used herein, the term "apolipoprotein B", refers to the polynucleotide and expression product e.g., polypeptide of the APOB gene. According to specific embodiments the apolipoprotein B refers to the human apolipoprotein B, such as provided in the following GeneBank Numbers NP_000375 and NM_000384 (SEQ ID NOs: 26-27). According to other specific embodiments, the apolipoprotein B refers to the mouse apolipoprotein B, such as provided in the following GeneBank Numbers NP_033823 and NM_009693 (SEQ ID NOs: 28-29).

By virtue of their anti-inflammatory activity, the polypeptides and compositions of matter of the present invention may be used to treat diseases which are dependent on CD44vRA (activity or expression) for their onset or progression, such as for the treatment of inflammatory diseases, such as Rheumatoid Arthritis (RA).

Thus, according to an aspect of the present invention there is provided a method of treating an inflammatory disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated polypeptide, the composition of matter or the pharmaceutical composition, thereby treating the inflammatory disease in the subject.

According to another aspect of the present invention there is provided a use of the isolated polypeptide, the composition of matter or the pharmaceutical composition for the manufacture of a medicament for the treatment of an inflammatory disease.

As used herein the term "treatiing" refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder, or condition e.g., inflammation e.g., RA) and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.
According to specific embodiments, the term "treating" refers to ameliorating symptoms associated with a RA and related diseases, lessening the severity or curing the diseases, or preventing the disease from occurring, preventing the manifestation of symptoms associated with the disease before they occur, slowing down the progression of the disease or deteriorating of the symptoms associated therewith, enhancing the onset of the remission period, slowing down the irreversible damage caused in the progressive chronic stage of the disease, delaying the onset of said progressive stage, improving survival rate or more rapid recovery, or a combination of two or more of the above.

As used herein the phrase "subject in need thereof" refers to a mammalian male or female subject (e.g., human being) who is diagnosed with an inflammatory disease or is at risk of to develop an inflammatory disease. Veterinary uses are also contemplated. The subject may be of any age including neonatal, infant, juvenile, adolescent, adult and elderly adult.

Methods of determining inflammation in a subject are well known in the art and include, but are not limited to, determining in a blood sample from the subject the erythrocyte sedimentation rate (ESR); plasma viscosity; levels of C-reactive protein (CRP); levels of certain inflammatory cytokines such as IL6 and TNFa; and determination of an inflammation index such as using fibrinogen measurements and hematocrit or hemoglobin.

According to specific embodiments, the inflammatory disease involves cells expressing CD44vRA. Non-limiting examples of assays that can evaluate the expression of CD44vRA on cells include flow cytometry and immunocytochemistry.

Examples of inflammatory diseases (also referred to herein as inflammation or inflammatory condition) include, but not limited to, chronic inflammatory disease and acute inflammatory disease.

Examples for Inflammatory disease include, but not limited to inflammatory diseases associated with hypersensitivity, autoimmune diseases, infectious diseases, graft rejection diseases, allergic diseases and cancerous diseases.

**Inflammatory diseases associated with hypersensitivity**

Examples of hypersensitivity include, but are not limited to, Type I hypersensitivity, Type II hypersensitivity, Type III hypersensitivity, Type IV...
hypersensitivity, immediate hypersensitivity, antibody mediated hypersensitivity, immune complex mediated hypersensitivity, T lymphocyte mediated hypersensitivity and DTH.

Type I or immediate hypersensitivity, such as asthma.


Type IV or T cell mediated hypersensitivity, include, but are not limited to, rheumatoid diseases, rheumatoid arthritis (Tisch R, McDevitt HO. Proc Natl Acad Sci U

Examples of delayed type hypersensitivity include, but are not limited to, contact dermatitis and drug eruption.
Examples of types of T lymphocyte mediating hypersensitivity include, but are not limited to, helper T lymphocytes and cytotoxic T lymphocytes.

Examples of helper T lymphocyte-mediated hypersensitivity include, but are not limited to, $T_{h1}$ lymphocyte mediated hypersensitivity and $T_{h2}$ lymphocyte mediated hypersensitivity.

Autoimmune diseases

Include, but are not limited to, cardiovascular diseases, rheumatoid diseases, glandular diseases, gastrointestinal diseases, cutaneous diseases, hepatic diseases, neurological diseases, muscular diseases, nephric diseases, diseases related to reproduction, connective tissue diseases and systemic diseases.


Examples of autoimmune rheumatoid diseases include, but are not limited to rheumatoid arthritis (Krenn V. et al., Histol Histopathol 2000 Jul;15 (3):791; Tisch R, McDevitt HO. Proc Natl Acad Sci units S A 1994 Jan 18;91 (2):437) and ankylosing spondylitis (Jan Voswinkel et al., Arthritis Res 2001; 3 (3): 189).

Examples of autoimmune gastrointestinal diseases include, but are not limited to, chronic inflammatory intestinal diseases (Garcia Herola A. et al, Gastroenterol Hepatol. 2000 Jan; 23 (1): 16), celiac disease (Landau YE. and Shoenfeld Y. Harefuah 2000 Jan 16; 138 (2): 122), colitis, ileitis and Crohn's disease.

Examples of autoimmune cutaneous diseases include, but are not limited to, autoimmune bullous skin diseases, such as, but are not limited to, pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus.


Examples of autoimmune neurological diseases include, but are not limited to, multiple sclerosis (Cross AH. et al, J Neuroimmunol 2001 Jan 1;112 (1-2):1).

Examples of autoimmune muscular diseases include, but are not limited to, myositis, autoimmune myositis and primary Sjogren's syndrome (Feist E. et al., Int Arch Allergy Immunol 2000 Sep;123 (1):92) and smooth muscle autoimmune disease (Zauli D. et al., Biomed Pharmacother 1999 Jun;53 (5-6):234).

Examples of autoimmune nephric diseases include, but are not limited to, nephritis and autoimmune interstitial nephritis (Kelly CJ. J Am Soc Nephrol 1990 Aug; 1 (2):140).

Examples of autoimmune diseases related to reproduction include, but are not limited to, repeated fetal loss (Tincani A. et al., Lupus 1998; 7 Suppl 2:S 107-9).

Examples of autoimmune connective tissue diseases include, but are not limited to, ear diseases, autoimmune ear diseases (Yoo TJ. et al., Cell Immunol 1994 Aug; 157 (1):249) and autoimmune diseases of the inner ear (Gloddek B. et al., Ann N Y Acad Sci 1997 Dec 29; 830:266).

Examples of autoimmune systemic diseases include, but are not limited to, systemic lupus erythematosus (Erikson J. et al., Immunol Res 1998;17 (1-2):49) and

**Infectious diseases**

Examples of infectious diseases include, but are not limited to, chronic infectious diseases, subacute infectious diseases, acute infectious diseases, viral diseases, bacterial diseases, protozoan diseases, parasitic diseases, fungal diseases, mycoplasma diseases and prion diseases.

**Graft rejection diseases**

Examples of diseases associated with transplantation of a graft include, but are not limited to, graft rejection, chronic graft rejection, subacute graft rejection, hyperacute graft rejection, acute graft rejection and graft versus host disease.

**Allergic diseases**

Examples of allergic diseases include, but are not limited to, asthma, hives, urticaria, pollen allergy, dust mite allergy, venom allergy, cosmetics allergy, latex allergy, chemical allergy, drug allergy, insect bite allergy, animal dander allergy, stinging plant allergy, poison ivy allergy and food allergy.

**Cancerous diseases**

Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma, and leukemia. Particular examples of cancerous diseases but are not limited to: Myeloid leukemia such as Chronic myelogenous leukemia. Acute myelogenous leukemia with maturation, Acute promyelocytic leukemia, Acute nonlymphocytic leukemia with increased basophils, Acute monocytic leukemia. Acute myelomonocytic leukemia with eosinophilia; Malignant lymphoma, such as Burkitt's Non-Hodgkin's; Lymphocytic leukemia, such as Acute lymphoblastic leukemia. Chronic lymphocytic leukemia; Myeloproliferative diseases, such as Solid tumors Benign Meningioma, Mixed tumors of salivary gland, Colonic adenomas; Adenocarcinomas, such as Small cell lung cancer, Kidney, Uterus, Prostate, Bladder, Ovary, Colon, Sarcomas, Liposarcoma, myxoid, Synovial sarcoma, Rhabdomyosarcoma (alveolar), Extraskeletal myxoid chondro sarcoma, Ewing's tumor; other include Testicular and ovarian dysgerminoma, Retinoblastoma, Wilms' tumor, Neuroblastoma, Malignant melanoma, Mesotheioma, breast, skin, prostate, and ovarian.
According to specific embodiments the inflammatory disease is selected from the group consisting of Rheumatoid arthritis (RA), psoriatic arthritis, Alzheimer's disease, cancer and cardiovascular disease.

According to other specific embodiments the inflammatory disease comprises RA.

As used herein, the phrase "rheumatoid arthritis (RA)" refers to an autoimmune disease which primarily affects the joints. RA includes, but is limited to, adult RA, juvenile idiopathic arthritis, juvenile RA and juvenile chronic arthritis. RA can be diagnosed according to the American Rheumatoid Association criteria for the classification of rheumatoid arthritis, or any similar criteria and includes active, early (active RA diagnosed for at least 8 weeks but no longer than four years) and incipient (polyarthritis that does not fully meet the criteria for a diagnosis of RA, in association with the presence of RA-specific prognostic biomarkers such as anti-CCP and shared epitope) RA.

Example of RA clinical parameters symptoms that can be monitored to indicate improvement during treatment with the isolated polypeptides and composition of matter of the invention are:

- Morning stiffness for at least one hour and present for at least six weeks;
- Swelling of three or more joints for at least six weeks;
- Swelling of wrist, metacarpophalangeal, or proximal interphalangeal joints for at least six weeks
- Symmetric joint swelling;
- Hand x-ray changes that include erosions or unequivocal bony decalcification;
- Rheumatoid subcutaneous nodules; and
- Rheumatoid factors

Additional parameters that can be used in human for assessing RA improvement can be according to the American College of Rheumatology (ACR) and include e.g. ACR improvement criteria -ACR20, ACR50 and ACR70 representing the percentage of disease activity improvement (20, 50 or 70 %) by the reduction in certain RA symptoms. Thus, for example, ACR20 refers to patients which achieve a 20 % improvement in tender and swollen joint counts and 20 % improvement in three of the five remaining ACR core set measures.
As the present inventors discovered 3 potential target proteins of the isolated polypeptides and have also shown that addition of one of these proteins (i.e. SAA) can prevent the in-vitro activity of the polypeptide (see Examples 7 and 9 and Figure 15 in the Examples section which follows) the present invention contemplates the use of a combined treatment comprising the isolated polypeptides or the compositions of matter of the present invention and serum amyloid A (SAA), thranthyretin and/or apolipoproteins B inhibitors.

Thus, according to another aspect of the present invention there is provided a method of treating an inflammatory disease in a subject in need thereof, the method comprising:

(a) administering to the subject a therapeutically effective amount of the isolated polypeptide or the composition of matter of some embodiments of the present invention; and

(b) administering to said subject a therapeutically effective amount of an inhibitor of a protein selected from the group consisting of serum amyloid A (SAA), thranthyretin and apolipoproteins B, thereby treating the inflammatory disease in the subject.

According to another aspect there is provided a use of the isolated polypeptide or the composition of matter of some embodiments of the present invention and an inhibitor of a protein selected from the group consisting of serum amyloid A (SAA), thranthyretin and apolipoprotein B for the manufacture of a medicament for the treatment of an inflammatory disease.

According to another aspect of the present invention there is provided an article of manufacture or a kit identified for use in treating inflammatory disease, comprising a packaging material packaging the isolated polypeptides or the composition of matter of some embodiments of the present invention and an inhibitor for a protein selected from the group consisting of SAA, thranthyretin and apolipoproteins B.

According to another aspect of the present invention there is provided a method of treating an inflammatory disease in a subject in need thereof, the method comprising:

(a) administering to the subject a therapeutically effective amount of an isolated polypeptide comprising an amino acid sequence selected from the group
consisting of SEQ ID NOs: 1-3, wherein said polypeptide comprises an anti-inflammatory activity; and

(b) administering to said subject a therapeutically effective amount of an inhibitor of a protein selected from the group consisting of serum amyloid A (SAA), thranthyretin and apolipoproteins B,

thereby treating the inflammatory disease in the subject.

According to another aspect there is provided a use of as isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3, wherein said polypeptide comprises an anti-inflammatory activity; and an inhibitor of a protein selected from the group consisting of serum amyloid A (SAA), thranthyretin and apolipoprotein B for the manufacture of a medicament for the treatment of an inflammatory disease.

According another aspect of the present invention there is provided an article of manufacture or a kit identified for use in treating inflammatory disease, comprising a packaging material packaging the isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3, wherein said polypeptide comprises an anti-inflammatory activity; and an inhibitor for a protein selected from the group consisting of SAA, thranthyretin and apolipoproteins B.

As used herein the term "inhibitor" refers to an agent which downregulates expression and/or activity of a protein (e.g. SAA, thranthyretin and apolipoprotein B) at the genomic (e.g. homologous recombination and site specific endonucleases) and/or the transcript level using a variety of molecules which interfere with transcription and/or translation (e.g., RNA silencing agents e.g. siRNA, shRNA, micro-RNA) or on the protein level (e.g., aptamers, small molecules and inhibitory peptides, antagonists, enzymes that cleave the polypeptide, antibodies and the like).

Non-limiting example include an antibody which inhibits SAA such as Anti-serum albumin A, an RNA interference targeted to SAA mRNA (see e.g. International Publication Application No: WO 2006071691), an antisense oligonucleotides targeted to apolipoprotein B, such as but not limited to Mipomersen (ISIS-301012, KYNAMRO™), Triazolones as apolipoprotein B synthesis inhibitors (see e.g. US Patent No. US 6197972) and an apolipoprotein B secretion inhibitor (see e.g. EP Application Publication No. EP 1099438).
According to a specific embodiment, step (a) is effected prior to step (b).

According to another specific embodiment, step (a) is effected following step (b).

According to yet another specific embodiment, step (a) is effected concomitantly with step (b).

Multiple rounds of administration according to the methods of the present invention and multiple doses of the isolated polypeptide or the composition of matter and the inhibitor can be administered. According to specific embodiments step (a) is effected multiple times. Thus, according to specific embodiments, administration of inhibitor is effected following at least one administration of the isolated polypeptide or the composition of matter. According to specific embodiments step (B) is effected multiple times. Thus, according to specific embodiments, administering the isolated polypeptide or the composition of matter of the present invention is effected following at least one administration of the inhibitor. According to specific embodiments, administering the isolated polypeptide or the composition of matter of the present invention is effected in a sequential order with administration of the inhibitor.

The isolated polypeptide or the composition of matter and the inhibitor may be packaged in the same container or in separate containers; each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the isolated polypeptide or the composition of matter and the inhibitor are in separate formulations.

According to other specific embodiments, the isolated polypeptide or the composition of matter and the inhibitor are in a co-formulation.

The isolated polypeptides, the compositions of matter and the inhibitors of the present invention can be provided to the subject per se, or as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

Thus, according to an aspect of the present invention there is provided a pharmaceutical composition comprising as an active agent the isolated polypeptide or the composition of matter; and a pharmaceutically acceptable carrier or diluent.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as
physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the polypeptide or composition of matter comprising the polypeptide accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, topical, intradermal, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

According to specific embodiments the route of administration is oral administration.

According to other specific embodiments, the route of administration is into the skin. Methods of administering an active agent into a skin are known in the art and include, for example, intradermal injections, gels, liquid sprays and patches which comprise the active agent and which are applied on the outer surface of the skin.

According to some embodiments of the invention, administration of the active agent into the skin of the subject is performed topically (on the skin).
According to some embodiments of the invention, administration of the active agent into the skin of the subject is performed non-invasively, e.g., using a gel, a liquid spray or a patch (e.g. reservoir type patch and matrix type patch) comprising the active ingredient, which are applied onto the skin of the subject.

It should be noted that in order to increase delivery of the active agent into the skin, the active agent can be formulated with various vehicles designed to increase delivery to the epidermis or the dermis layers. Such vehicles include, but are not limited to liposomes, dendrimers, noisome, transfersome, microemulsion and solid lipid nanoparticles.

According to some embodiments of the invention, administering the is performed by an intradermal injection.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient such as a local injection into the joint. Methods of administering an active agent into the joint are known in the art and include intra-
articul injection wherein a hypodermic needle is inserted into the joint to thereby deliver the active agent to the intra-articular space of the intra-articular joint.

As described the polypeptides and compositions of matter of the invention may be used to treat e.g. Alzheimer's disease. Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intrahippocampal (IH), intracranial (IC), intracerebral injection, intracerebroventricular injection (ICV) or infusion or intrathecal administration); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.
For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

According to specific embodiments, the pharmaceutical composition is formulated for oral administration.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients
may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.
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The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical composition of some embodiments of the invention may also be formulated for sustained-release to provide elevated serum half-life. Such sustained release systems are well known to those of skill in the art and include e.g. microcapsules and nanoparticles. According to specific embodiments, the ProLease biodegradable microsphere delivery system for proteins and peptides (Tracy, 1998, Biotechnol. Prog. 14, 108; Johnson et al., 1996, Nature Med. 2, 795; Herbert et al., 1998, Pharmaceut. Res. 15, 357) a dry powder composed of biodegradable polymeric microspheres containing the protein in a polymer matrix that can be compounded as a dry formulation with or without other agents.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., RA) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can
be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.l).

Dosage amount and interval may be adjusted individually to provide that the levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

The doses shown herein with respect to the mouse animal model can be converted for the treatment other species such as human and other animals diagnosed with the inflammatory disease. Conversion Table approved by the FDA is shown in Reagan-Shaw S., et al., FASEB J. 22:659-661 (2007).

The human equivalent dose is calculated as follows: HED (mg/kg) = Animal dose (mg/kg) multiplied by (Animal $K_m$/human $K_m$).

According to some embodiments of the invention, the isolated polypeptide or the composition of matter is provided at an amount equivalent to a range of from about 2.5 - 40 mg/kg/day in mice, including any intermediate subranges and values therebetweeen.

According to specific embodiments the isolated polypeptide or the composition of matter is provided at an amount equivalent to about 3.5 mg/kg/day in mice.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser
may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

It will be appreciated that the therapeutic agents of the present invention can be provided to the individual with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In such therapy, measures (e.g., dosing and selection of the complementary agent) are taken to adverse side effects which may be associated with combination therapies.

Administration of such combination therapy can be simultaneous, such as in a single capsule having a fixed ratio of these active agents, or in multiple capsules for each agent.

Thus, the agents of the present invention can be administered alone with other established or experimental therapeutic regimen to treat inflammatory disease such as nonsteroidal anti-inflammatory drugs (NSAID), disease-modifying antirheumatic drugs (DMARDS), corticosteroids, analgesics, Fibromyalgia medications, chemotherapeutic agents and other treatment regimens which are well known in the art.

As mentioned, the present inventors have developed a novel in-vitro assay to test the activity of the peptides and compositions of matter of the present invention. The assay is based on the realization that the RA peptides can reduce survival of fibroblasts isolated from the synovial fluid of an RA patient. Thus, this assay can be used to compare batch to batch variation of manufactured peptides and compositions of matter peptides of the present invention for qualifying the anti-inflammatory activity as well as for testing e.g. stability of the peptides and compositions of matter of the present invention following exposure to environmental conditions such as storage temperature, modifications to the peptides and the formulations.
Thus, according to an aspect of the present invention there is provided a method of determining potency of a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition of some embodiments of the present invention, the method comprising:

(a) contacting a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition with fibroblasts obtained from an inflammatory joint of a Rheumatoid arthritis patient; and

(b) determining survival of said fibroblasts following a predetermined incubation time, so as to determine the potency of the batch.

According to specific embodiments, the method comprising synthesizing the isolated polypeptide, the composition of matter or the pharmaceutical composition with a modification. Such a modification can be any of the modifications presented hereinabove.

According to specific embodiments, the method is effected in-vitro or ex-vivo.

As used herein, the term "potency" refers to the measure of the biological activity of the product (i.e.; the isolated polypeptide or the composition of matter), based on the attribute of the product which is linked to the relevant biological properties (i.e.; reduced survival of fibroblasts obtained from an RA patient).

As used herein, the term "batch" refers to a specific quantity of a drug that is intended to have uniform character and quality, within specified limits, and is typically produced according to a single manufacturing order during the same cycle of manufacture. Thus, the present teachings can be used in the QA of the manufacturing procedures for assessing the biological activity of the isolated polypeptides, the composition of matter or the pharmaceutical compositions as part of batch qualification.

According to specific embodiments, the term "batch" also refers to a quantity of the drug exposed to stability characterization and/or peptide and formulation modifications.

As used herein, the term "fibroblast" refers to a connective tissue cell that synthesizes the extracellular matrix and collagen and is obtained from a synovial fluid of an RA patient. The fibroblasts used according to the method can be a primary culture directly isolated from an RA patient or cell lines obtained from the fibroblasts such as
the commercially available cell lines SW 982, PCS-201-010 and ACS-1023 that can be obtained from the ATCC.

Methods for obtaining a synovial fluid from a subject are well known in the art and include, but are not limited to biopsy such as joint biopsy and joint aspiration. Typically, procedures for obtaining tissue or fluid biopsies are described in details in Hypertext Transfer Protocol://World Wide Web (dot) healthatoz (dot)com/healthatoz/Atoz/search.asp.

Specifically, a joint aspiration, also known as Arthrocentesis refers to the removal of fluid from the space around a joint using a needle and syringe. This is usually performed under a local anesthetic to either relieve swelling or to obtain fluid for analysis to diagnose a joint disorder and/or problem. Joint aspiration is usually performed on the knee; however, fluid can also be removed from other joints, such as the hip, ankle, shoulder, elbow, or wrist.

A joint biopsy refers to joint or synovial biopsy. In the procedure a sample of the joint lining or synovial membrane or fluid is taken. Briefly, the procedure is effected in a clinical facility by a surgeon. A number of approaches are available to perform this biopsy: such as through an incision in the joint; with a scope inserted in the joint; or, more typically, by the insertion of a sharp instrument through the skin. The sample can be taken from any joint, typically the examined joint is the knee. A sharp instrument (trocar) is pushed into the joint space. A needle with an attached syringe is inserted into the joint to withdraw fluid for laboratory analysis. The surgeon may instill analgesic compounds into the joint and along the needle track before the needle is withdrawn. The trocar and then the biopsy needle is inserted and specimens taken. After the specimen is taken, both the trocar and the biopsy needle are removed.

Regardless of the procedure employed, once the biological sample is obtained, the fibroblast may be further isolated. Enrichment of fibroblasts populations can be obtained by methods well known in the art, and included those disclosed in e.g. Bendersky et al, J Immunol.;188:4349-59, 2012, magnetic cell separation and flow cytometry cell sorting.

Thus, for example the fibroblasts can be isolated by culturing adherent synovial fluid cells in plastic wells in DMEM-supplemented medium for about 48 hours followed by removal of all of the nonadherent cells. The adherent cells which comprise
fibroblasts typically display a fibroblastic morphology and express CD90, low levels of the CD49a integrin, negative for macrophage (CD14), T cell (CD3), and B cell (CD20) surface markers (as determined by e.g. flow cytometry) and stain positively for collagen type I determined by Sirius red staining.

The primary culture of fibroblasts comprises according to specific embodiments at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more fibroblasts.

Following preparation of the fibroblasts, predetermined amount of cells are incubated in tissue culture plates (e.g.; 12, 24, 96, 384 wells plates) with the appropriate Growth medium and stimulated with a predetermined amount of the tested isolated polypeptide, the composition of matter or the pharmaceutical composition. Selection of the medium is well within the capabilities of skilled in the art. Thus, for example, RPMI or DMEM (can be obtained for example from Sigma-Aldrich or Biological Industries, Beit Haemek, Israel) can be used as a growth medium. The medium may be supplemented with L-glutamine, non-essential amino acids, sodium pyruvate, antibiotic/antimycotic solution, 2-mercaptoethanol and serum.

Selection of the predetermined amount of cells incubated for in-vitro testing that will result in detectable effect on cell survival is well within the capabilities of the skilled in the art. Thus, for example cell concentration can be 1x10^4 / ml to 5x10^6 / ml; 1x10^4 / ml to 1x10^6 / ml; 5x10^4 / ml to 5x10^6 / ml; 1x10^5 / ml to 5x10^6 / ml; or 1x10^5 / ml to 1x10^6 / ml.

According to specific embodiments the cell concentration is 2x10^5 / ml.

Selection of the peptide or composition of matter concentration used for the in-vitro testing that will result in detectable effect on cell survival is well within the capabilities of skilled in the art. Preferably, the concentration used should be within the linear range of the selected stimulation parameter. Thus, for example, the concentration can be 1 µg / ml to 30 µg / ml; 1 µg / ml to 20 µg / ml; 5 µg / ml to 30 µg / ml; or 5 µg / ml to 20 µg / ml.

The number of tested concentration can be at least 1, at least 2, at least 3, at least 5, at least 6, 1-10, 2-10, 3-10, 5-10, 1-5, 2-5 and 3-5 different concentrations.

The number of samples repeats for each of the tested concentration can be 2, 3, 4, 5 or 6 repeats.
Following a pre-determined incubation time the survival of the fibroblasts is determined. Specific methods of monitoring cell survival are known in the art and include for example, the MTT test which is based on the selective ability of living cells to reduce the yellow salt MTT (3-(4, 5- dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (can be obtained for example from Sigma, Aldrich) to a purple-blue insoluble formazan precipitate; Apoptosis assays such as the TUNEL assay (can be obtained for example from Roche); and the Annexin V assay [for example ApoAlert® Annexin V Apoptosis Kit (Clontech Laboratories, Inc., CA, USA)].

The incubation time may vary and determination of the incubation time that will result in detectable effect on cell survival is well within the capabilities of skilled in the art. According to a specific embodiment, the incubation time is between 12 hours to 96 hours. According to some embodiments of the invention, the incubation time is between 12 to 72 hours; 12 to 48 hours; 12 to 24 hours; 24 to 96 hours, 24 to 72 hours or 24 to 48 hours. According to specific embodiments of the invention, the incubation time is between 24-48 hours.

According to specific embodiments, reduced survival of the fibroblasts following said contacting is indicative that the batch is potent.

According to specific embodiments the assay may further include positive and negative control samples. The positive control for the assay may include agents inducing non-specific fibroblasts cell death, for example ascorbate (see e.g. Schmidt et al., J Biomed Mater Res. 1993 Apr; 27(4):521-30).

Negative control for the assay may include agents which prevent the biological activity of the tested polypeptide or composition of matter such as SAA (can be obtained for example from PeproTech).

According to specific embodiments, the method comprising comparing the survival of the cells with survival of the cells following contacting with a reference standard batch of the isolated polypeptide, composition of matter or the pharmaceutical composition, so as to determine the relative potency of the batch.

As used herein, the term "relative potency" refers to a qualitative measure of potency of a batch of the isolated polypeptide, composition of matter or the pharmaceutical composition, relatively to a standard reference (RS) of the isolated
polypeptide, composition of matter or the pharmaceutical composition, having a known potency.

According to specific embodiments the potency of a batch of the isolated polypeptide, composition of matter or the pharmaceutical composition, is determined relatively to the known potency of a reference standard (RS).

As used herein, the phrase "reference standard" or "RS" refers to a standardized isolated polypeptide, composition of matter or pharmaceutical composition, which is used as a measurement base for the isolated polypeptide, composition of matter or the pharmaceutical composition. RS provides a calibrated level of biological effect against which new preparations of the isolated polypeptide, composition of matter or the pharmaceutical composition can be compared to.

According to a specific embodiment, the RS is characterized by optimum potency and quality of an active component that is effective in treating the disease (e.g., RA).

Calculating potency and relative potency are known in the art. According to specific embodiments the relative potency is calculated using a software suitable for biological assays, such as parallel line analysis software e.g., PLA (Stegmann Systems GmbH) and Gen5 data analysis software (BioTek).

Implementation of the method and/or system of embodiments of the invention can involve performing or completing selected tasks manually, automatically, or a combination thereof. Moreover, according to actual instrumentation and equipment of embodiments of the method and/or system of the invention, several selected tasks could be implemented by hardware, by software or by firmware or by a combination thereof using an operating system.

As used herein the term "about" refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.
As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in
1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

**EXAMPLES**

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.


EXAMPLE 1

PEPTIDE SYNTHESIS AND CHARACTERIZATION

Materials and Methods

Synthesis of 5, 7 and 9 mer peptides - The 5-, 7- and 9-mer peptides [MTADV (SEQ ID NO: 1), MTADVDR (SEQ ID NO: 2) and TRMTADVDR (SEQ ID NO: 3)], 3, the Acylated-N and Amidated-C termini 5-, 7- and 9-mer peptides [Ac-MTADV-NH2 (SEQ ID NO: 4), Ac-MTADVDR-NH2 (SEQ ID NO: 5) and Ac-TRMTADVDR-NH2 (SEQ ID NO: 6)] and the scrambled 7-mer peptide [Ac-TMDVADR-NH2 (SEQ ID NO: 7)], were synthesized by Sigma Israel using solid phase synthesis fmoc chemistry. A purity of 95- to 97 % was reached.

Liquid chromatography-mass spectrometry (LCMS) - Stability, Pharmacokinetic (PK) and target proteins of the peptide were evaluated by LCMS.
Sample preparation for mass spectrometry: The protein-bound beads were reduced with 2.8mM DTT (60 °C for 30 min) and modified with 8.8 mM iodoacetamide (at room temperature for 30 min under dark conditions) in 8M Urea and 400 mM Ammonium bicarbonate. The proteins were digested in 2M Urea, 25 mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37 °C. The tryptic peptides were desalted using C18 tips (Harvard) dried and re-suspended in 0.1 % Formic acidMass Spectrometry measurements. The resulting tryptic peptides were analyzed by LC-MS/MS using an OrbitrapXL mass spectrometer (Thermo-Fisher) fitted with a capillary HPLC (Eksigent). Specifically, the peptides were loaded onto a C18 trap column (0.3 5mm, LC-Packings) connected on-line to a homemade capillary column (75 micron ID) packed with Reprosil C18-Aqua (Dr Maisch GmbH, Germany) and resolved using linear 94 minutes 5 to 40% acetonitrile gradients followed by 12 minutes at 95 % acetonitrile in the presence of 0.1 % formic acid in water at flow rates of 0.25 μl/min. Mass spectrometry was performed in a positive mode using repetitively full MS scan (resolution 60000) followed by collision induces dissociation (CID). Top seven, (>1 charged peptides, 350-2000 M/Z) were selected for fragmentation from each full mass spectrum.

Data analysis: The mass spectrometry was analyzed using the Discoverer software version 1.4 against the human uniprot database and against decoy databases (in order to determine the false discovery rate (FDR), using the Sequest and Mascot search engines. High confidence refers to 0.01 FDR .

Semi quantitation was performed by calculating the peak area of each peptide. The area of the protein is the average of the three most intense peptides from each protein.

Results

Inclusion of alanine in the splicing junction between variant exon 4 and variant exon 5 of CD44vRA variant leading to the presence of the MTADV sequence instead of the original MTDV sequence was shown to confer the pathological activity of CD44vRA (Nedvetzki et al., J Clin Invest 111:121 1-1220, 2003).

The present inventors have synthesized 5-, 7-, and 9-mer peptides (denoted herein as RA peptides) including the MTADV sequence. The 5-mer RA peptide comprises a relative hydrophobic amino acid sequence as well as a low molecular weight (less than 700
Dalton). Therefore, it may easily penetrate into tissues and consequently may be used in topical applications and oral delivery.

The proteolytic analysis of the RA peptides demonstrated no proteolytic sites (data not shown), indicating that the peptides are at least relatively stable. Quantification analysis by Mass Spectrometry indicated almost identical stability of the 5-mer peptide (SEQ ID NO: 1) upon storage in saline at 4 °C, at room temperature or at -20 °C for 22 weeks (Figure 1). Assuming that storage at -20 °C displays 100 % stability, these results demonstrate that the 5-mer peptide is stable for at least 22 weeks at room temperature and 4 °C.

EXAMPLE 2
THE 5- and 9-MER RA PEPTIDES CAN REDUCE JOINT INFLAMMATION IN THE COLLAGEN-INDUCED ARTHRITIS (CIA) MOUSE MODEL

Materials and Methods

Mice - Collagen-induced arthritis (CIA) was generated in DBA/1 or C57BL mice by injection of type II collagen as described in Nedvetzki et al., PNAS 101, 18081-18086, 2004.

Treatment protocol - PBS, or the 9-mer RA peptide (SEQ ID NO: 3) at a dose of 25 µg, 100 µg or 150 µg per injection was administered i.p to CIA mice for 4 times within 6 days (see Table 3 below). PBS (n = 7), or 5-mer RA peptide (SEQ ID NO: 1, n = 7) at a dose of 70 µg per injection were administered i.p to CIA mice on C57BL/6 background for 10 consecutive days. In all groups first injection was given at the onset of disease as determined by paw swelling.

Evaluation of joint inflammation - Inflammation was evaluated by the paw swelling response. Paw swelling was measured by micro caliber. Paw swelling at range of 2.1-2.3 mm was considered "onset of disease" and the starting point for injection of the peptides. Mice that showed above 2.3 mm paw swelling were excluded from the experiment. All measurements were performed under blind manner (For additional details see Nedvetzki, et al., (2004) PNAS 101, 18081-18086).

Histology - Mice were sacrificed on day 11 (one day following cessation of treatment) and hind limbs were isolated and fixed overnight with 4 % paraformaldehyde at room temperature (for additional details see Nedvetzki et al. (2004) PNAS 101,18081-18086). Following fixation, diarthrodial Joint sections were prepared, stained with
Hematoxylin and Eosin (H&E) and evaluated by a pathologist under blind manner. Joint infiltration score: 0 = no infiltration; 4 = massive infiltration.

**Statistical analysis** - Statistical analysis for each measurement point was performed by the Student's t-test for unpaired values.

<table>
<thead>
<tr>
<th>Table 3: Experimental design- dose response (Figures 2A-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group #</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

**Results**

The collagen-induced arthritis (CIA) mouse model is the mouse analogue of human Rheumatoid Arthritis (RA). To evaluate the effect of the generated RA peptides on joint inflammation in the CIA mouse model different dosages of the 9-mer RA peptide (SEQ ID NO: 3) were administered to CIA mice on DBA/1 background starting from the onset of disease; and joint inflammation was evaluated by determining paw swelling. As shown in Figures 2A-B, mice that received 25 and 100 µg of the 9-mer RA peptide (SEQ ID NO: 3) did not show significant difference in paw swelling response as compared to mice administered with PBS, implying lack of a significant therapeutic effect. In contrast, mice that received 150 µg of the 9-mer RA peptide (SEQ ID NO: 3) had demonstrated significant reduction in paw swelling (Figure 2C).

Histological evaluation of hind limbs sections taken from CIA mice on C57BL/6 background following treatment with the PBS control revealed massive infiltration of mononuclear inflammatory cells into the joint capsule, extensive synovial hypertrophy and severe narrowing of the joint space which was filled with reactive cells. In addition, severe erosion of the articular cartilage and the subchondral bone, with destruction of cartilage matrix, was noted. The remaining cartilage matrix was heavily infiltrated as well. Taken together, the average joint Infiltration score in the PBS treated mice = about 4 (Figures 3A and 3C).

On the contrary, histological evaluation of hind limbs sections taken from CIA mice following treatment with the 5-mer RA peptide (SEQ ID NO: 1) revealed none-to-mild infiltration of mononuclear inflammatory cells into the joint capsule with either no
or mild synovial hypertrophy. Inflammatory infiltrate was noted in minority of the samples. The joint space was preserved, with few reactive cells if any. In addition, no erosion of the articular cartilage and the subchondral bone was noted and the cartilage matrix was generally preserved. Furthermore, few of the samples were indistinguishable from unaffected mice (data not shown). Taken together, average joint infiltration score in the 5-mer RA peptide treated mice = about 1 (Figures 3B and 3C).

Overall, the joint inflammation score of the control PBS treated group was significantly higher (P<0.0001) than that of the 5-mer RA peptide (SEQ ID NO: 1) treated group, indicating that treatment with the peptide can highly restore normal histology of the inflamed joint.

Taken together, treatment with the 5- and 9-mer RA peptides (SEQ ID NOs: 1 and 3) inhibit joint inflammation in the CIA mouse model.

Without being bound by theory it is suggested that an interaction between unknown CD44vRA ligand and CD44vRA create a conformational change of CD44vRA glycoprotein. This new epitope allows binding of FGF-2 to the heparin sulfate of v3 exon product resides in the same molecule. The bound FGF-2 is oriented to interact with endothelial cells or fibroblasts expressing FGF receptor 1, resulting in their proliferation and exaggeration of the inflammatory activity (Nedvetzki et al., J Clin Invest 111:1211-1220, 2003). The RA peptide may compete with the cell surface CD44 on the interaction with the unknown ligand resulting in blockade of the FGF-2-induced inflammatory cascade described by Nedvetzki et al. (J Clin Invest 111:1211-1220, 2003).

**EXAMPLE 3**

**THE 5-7- AND 9-MER PROTECTED RA PEPTIDES CAN REDUCE JOINT INFLAMMATION IN THE CIA MOUSE MODEL**

**Materials and Methods**

*Mice* - As described in Example 2 above.

*Treatment protocol* - PBS, or the tested peptides [Ac-TRMTADVDR-NH2 (SEQ ID NO: 6), Ac-MTADVDR-NH2 (SEQ ID NO: 5), Ac-MTADV-NH2 (SEQ ID NO: 4) and scrambled Ac-TMDVADR-NH2 (SEQ ID NO: 7)] at doses of 70 or 200 µg per injection were administered i.p to CIA /1 mice on DBA background for 9-10 consecutive days (see Tables 4-7 below). As a non-specific control, several mice were
administered I.P. with Dexamethsone (Dex) at a dose of 50 µg, following the same protocol. In all groups first injection was given at the onset of disease as determined by paw swelling.

*Evaluation of joint inflammation*- As described in Example 2 above. Paw swelling of less than 2mm was considered healthy.

*Statistical analysis*- As described in Example 2 above.

**Table 4: Experimental design (Figure 4)**

<table>
<thead>
<tr>
<th>Group #</th>
<th>Treatment</th>
<th>#Mice/paws</th>
<th>Injection protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBSx1 100ul/injection</td>
<td>4/5</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>Peptide 9 mer Ac-TRMTADVDR-NH2 (SEQ ID NO: 6) 200µg/100µl/injection</td>
<td>5/7*</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>3</td>
<td>Peptide 7 mer Ac-MTADVDR-NH2 (SEQ ID NO: 5) 200µg/100µl /injection</td>
<td>2/3*</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
</tbody>
</table>

*When the two hind paws of a mouse were inflamed, both paws were analyzed for swelling

**Table 5A: Experimental design (Figure 5A)**

<table>
<thead>
<tr>
<th>Group #</th>
<th>Treatment</th>
<th>#Mice</th>
<th>Injection protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1XPBS 100ul/injection</td>
<td>3</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>Peptide 5 mer Ac-MTADV-NH2 (SEQ ID NO: 4) 200µg/100µl/injection</td>
<td>5</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>3</td>
<td>Dexamethasone (Dex)</td>
<td>4</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
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</table>

**Table 5B: Experimental design (Figure 5B)**

<table>
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<tr>
<th>Group #</th>
<th>Treatment</th>
<th>#Mice/Paws*</th>
<th>Injection protocol</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1XPBS 100µl/injection</td>
<td>10/11*</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>Peptide 5 mer Ac-MTADV-NH2 (SEQ ID NO: 4) 200µg/100µl/injection</td>
<td>11/16*</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
</tbody>
</table>

*When the two hind paws of a mouse were inflamed, both paws were analyzed for swelling

**Table 6: Experimental design (Figure 6)**

<table>
<thead>
<tr>
<th>Group #</th>
<th>Treatment</th>
<th>#Mice</th>
<th>Injection protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1XPBS 100µl/injection</td>
<td>13</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>Peptide 5 mer Ac-MTADV-NH2 (SEQ ID NO: 4) 70µg/100µl/injection</td>
<td>11</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
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</table>

**Table 7: Experimental design (Figures 7A-C)**

<table>
<thead>
<tr>
<th>Group #</th>
<th>Treatment</th>
<th>#Mice/Paws*</th>
<th>Injection protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1XPBS 100µl/injection</td>
<td>8/9</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>Control: 7-mer scrambled Peptide Ac-TMDVADR-NH2 (SEQ ID NO: 7) 200µg/100µl /injection</td>
<td>7/10*</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
<tr>
<td>3</td>
<td>Peptide 7-mer Ac-MTADVDR-NH2 (SEQ ID NO: 5) 200µg/100µl/injection</td>
<td>9/11*</td>
<td>One injection per day for 9 constitutive days</td>
</tr>
</tbody>
</table>

*When the two hind paws of a mouse were inflamed, both paws were analyzed for swelling
Results

In the next step 9- 7- and 5-mer RA peptides were synthesized with protection residues, namely Acetyl and Amide residues at the amino and carboxyl terminal ends of the peptides [Ac-TRMTADVDR-NH₂ (SEQ ID NO: 6), Ac-MTADVDR-NH₂ (SEQ ID NO: 5) and Ac-MTADV-NH₂ (SEQ ID NO: 4), denoted herein as 9- 7- and 5- mer RA protected peptide, respectively]. These protection residues preserve the natural stage of the peptide in the experimental mouse and stabilize the peptide. The ability of the protected peptides to reduce joint inflammation in DBA/1 mice following their injection at the onset of CIA was evaluated. All measurements were performed under blind manner.

As can be seen in Figures 4 and 5A-B injection of all protected RA peptides at a dose of 200 µg per injection significantly inhibited joint inflammation in the CIA mice on DBA background, as compared to mice treated with PBS. As also evident in Figure 5A, administration of Dexamethasone decreased footpad swelling as well, possibly by generating a non-specific anti-inflammatory effect of this steroid. The 5-mer protected peptide (SEQ ID NO: 4) was also able to significantly inhibit joint inflammation in the CIA mice on C57BL/6 background when administered at a dose of 70 µg per injection (Figure 6). All measurements were performed under blind manner.

In the next step, the effect of a scrambled RA peptide on joint inflammation was evaluated. To this end the effect of the 7-mer protected RA peptide (Ac-MTADVDR-NH₂, SEQ ID NO: 5) was compared to the effect of a 7-mer scrambled protected peptide (Ac-TMDVADR-NH₂, SEQ ID NO: 7). As shown in Figures 7A-C, the scrambled non-specific 7-mer protected peptide had no effect on footpad swelling. All measurements were performed under blind manner.

The results shown in Figures 7A-C were also evaluated by determining the percent of healthy paws in each group. Hence, evaluation of the hind paws of the CIA DBA/1 mice (Figure 8), reflecting the severity of the disease, showed that more than 60% of the hind paws in CIA mice treated with the 7-mer protected RA peptide remained healthy. In comparison, the percentages of the hind paws that remained healthy in the other groups tested were 15% in the PBS treated group and 33% in the 7-mer peptide scrambled group.
Taken together, 5-, 7- and 9-mer RA protected peptides (SEQ ID NOs: 4-6) inhibit joint inflammation upon injection to CIA mice on both DBA and C57BL/6 background, while non-specific scrambled 7-mer protected peptide (SEQ ID NO: 7) has no effect on joint inflammation. Acetylation and Amidation did not affect the activity of the RA peptides (data not shown); however, they are expected to improve stability and pharmacokinetics of the RA peptide.

EXAMPLE 4

70 µg PER INJECTION IS THE OPTIMAL DOSE FOR THE 5-MER PROTECTED RA PePTIDE FOR REDUCING JOINT INFLAMMATION IN THE CIA MOUSE MODEL

Materials and Methods

Mice - As described in Example 2 above.

Treatment protocol - PBS, or the tested peptide [Ac-MTADV-NH₂ (SEQ ID NO: 4)] at doses of 10, 25, 70, 200 or 600 µg per injection were administered i.p to CIA mice for 10 consecutive days (see Tables 8-9 below). In all groups first injection was given at the onset of disease as determined by paw swelling.

Evaluation of joint inflammation - As described in Example 2 above. The measurements were effected under blind manner.

Statistical analysis - As described in Example 2 above.

Table 8: Experimental design (Figure 9A)

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<th>Treatment</th>
<th># Paws</th>
<th>Injection protocol</th>
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<tr>
<td>1</td>
<td>1XPBS 100µl/injection</td>
<td>13</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 70µg/100µl / injection</td>
<td>11</td>
<td>One injection per day for 10 constitutive days</td>
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<td>3</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 200µg/100µl / injection</td>
<td>11</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>4</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 600µg/100µl / injection</td>
<td>9</td>
<td>One injection per day for 10 constitutive days</td>
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Table 9: Experimental design (Figure 9B)

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<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>2</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 10µg/100µl / injection</td>
<td>11</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
<tr>
<td>3</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 25µg/100µl / injection</td>
<td>11</td>
<td>One injection per day for 10 constitutive days</td>
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<tr>
<td>4</td>
<td>5 mer Peptide Ac-MTADV-NH₂ (SEQ ID NO: 4) 70µg/100µl / injection</td>
<td>9</td>
<td>One injection per day for 10 constitutive days</td>
</tr>
</tbody>
</table>
Results

To evaluate the optimal anti-inflammatory therapeutic dose of the 5-mer RA peptide, several different doses of the protected peptide (SEQ ID NO: 4) were administered to CIA mice on C57BL/6 background and joint inflammation was determined by footpad swelling measurements (electronic automatic measurements of the volume of the footpad, based on Archimedes observation). As can be seen in Figure 9A, injection of 70, 200 or 600 µg per injection of the 5-mer protected RA peptide (SEQ ID NO: 4) reduced the joint inflammation when compared to PBS control; however, a dose of 70 µg of the peptide generated the most statistically significant anti-inflammatory effect. In addition, as shown in Figure 9B, injection of 10 and 25 µg per injection of the 5-mer protected RA peptide (SEQ ID NO: 4) did not induce a significant anti-inflammatory effect.

Taken together, the data indicates that a dose of 70 µg per injection is the optimal and the lowest dose for CIA inhibition by the 5-mer protected RA peptide (SEQ ID NO: 4).

EXAMPLE 5

THE 5-MER RA PEPTIDE DOES NOT INHIBIT DTH IN THE CIA MOUSE MODEL

Materials and Methods

DTH model - C57BL/6 mice were painted at their abdomen with oxazolone solution (sensitization). On day 6, the right ear of each mouse was painted with the same hapten, oxazolone (elicitation), to generate delayed type hypersensitivity (DTH) response. The differences in thickness between the right and the left ears, indicating DTH development, were determined by microcaliper 24 hours later. DTH induction indicates a normal immune response. For additional details see Weiss et al., (2000) Proc. Natl. Acad. Sci. USA. 97, 285-290.

Treatment protocol - PBS and 5-mer peptide (SEQ ID NO: 1) at a dose of 200 µg was administered one day before the sensitization and then every day during the sensitization period (7 days). An anti-TNF antibody (Herrring at al., (2002) Infect Immun 70, 2959-64) was used for comparison, to demonstrate non-specific effect.

Statistical analysis - As described in Example 2 above.
Results

To evaluate the influence the RA peptides on the immune response in general the effect of the 5-mer RA peptide (SEQ ID NO: 1) on delayed type hypersensitivity (DTH) response was evaluated in a DTH model generated in C57BL/6 mice. DTH reflects acute inflammation, characterizing immune response against microorganism. The results indicate that injection of the 5-mer RA peptide did not affect the DTH response and mice treated with the peptide displayed the same DTH response as the control mice treated with PBS, throughout the 7 days assay period (Figure 10). In comparison mice treated with anti-TNFα antibody displayed inhibited DTH response, which was significant compared to the control mice.

EXAMPLE 6

THE 5-MER PEPTIDE DOES NOT GENERATE NEUTRALIZING ANTIBODIES IN THE CIA MOUSE MODEL

Materials and Methods

Mice - As described in Example 2 above.

Treatment protocol - PBS, or 5-mer RA peptide (SEQ ID NO: 1) at a dose of 70 µg per injection were administered i.p to CIA mice on C57BL/6 background for 10 consecutive days. In all groups first injection was given at the onset of disease as determined by paw swelling.

ELISA - 96 wells ELISA plates were coated with 5-mer RA peptide (SEQ ID NO: 1), collagen or immunoglobulin (positive control). Serum from CIA mice treated with PBS or the 5-mer RA peptide was added to the coated plates and the presence of neutralizing antibodies against collagen or the RA peptide was detected with anti-immunoglobulin + detection system. The plate wells were coated with 1 mg/ml peptide or protein. Mouse serum was added to the plate wells for 15 hours in cold temperature. The detection system included HRP-anti-mouse IgG and TMB (Bako) substrate. Plates were analysed using an ELISA reader at a wave length of 450 nm.

Statistical analysis - As described in Example 2 above.

Results

To determine whether treatment with the RA peptides induces production of specific neutralizing antibodies that may reduce or even block the peptide anti-inflammatory effect the abundance of anti-5-mer peptide antibodies in the serum was
63
determined using an ELISA assay. As shown in Figure 11, no neutralizing antibodies to
the 5-mer RA peptide (SEQ ID NO: 1) were detected in the serum of CIA mice following
treatment with the peptide. Contrary, as the CIA mouse model is generated by collagen
injection, anti-collagen specific antibodies were clearly evident in the serum of the CIA
mice.

EXAMPLE 7
SERUM AMYLOID A, TRANSTHYRETIN AND APOLIPOPROTEIN B ARE
POTENTIAL TARGET PROTEINS OF THE 5-MER PEPTIDE

Materials and Methods

Separation of peptide target protein(s) - Synovial fluid was removed from the
joint of a Rheumatoid Arthritis (RA) patient. The synovial fluid was diluted 1:1 with
PBS and centrifuged at 1,200 rpm. The cell pellet was subjected to lysis buffer containing
protease inhibitors. The cell lysate was incubated with a biotinylated 5-mer peptide
(Sigma) or with PBS for 12 hour at 4 °C, with shaking. Streptavidin Sepharose beads
(Sephdex) were added to the biotinylated 5-mer peptide-treated cell extract or PBS-
treated cell extract for additional one hour at 4 °C, with shaking. The peptide-bound
beads and the control beads were separated by centrifugation, extensively washed and
sent for mass spectrometry analysis, see Figure 12. The mass spectrometry (MS)
measurements and analysis were performed in the Smoler Proteomic Research Center at
the Technion in Haifa.

Results

Mass spectrometry analysis of proteins from cell lysates extracted from synovial
fluid cells of an RA patient that bound the 5-mer peptide (SEQ ID NO: 1) identified
Serum Amyloid A (SAA), Transthyretin and Apolipoprotein B as potential target proteins
of the 5-mer RA peptide (SEQ ID NO: 1). The indicated proteins are known to be
involved in the pathology of RA but also in the pathologies of Alzheimer's disease,
cancer diseases and cardiovascular disease.

EXAMPLE 8
PHARMACOKINETICS OF THE 5-MER RA PEPTIDE

Materials and Methods

Treatment protocol - C57BL/6 mice were subjected to a single i.p. injection of
200 µg 5-mer peptide (SEQ ID NO: 1). Blood samples were taken by terminal bleeding
Mass Spectrometry analysis - The concentration of the 5-mer RA peptide in the blood was determined by mass spectrum analysis as described above.

Results

The pharmacokinetic (PK) of the 5-mer RA peptide elimination in the blood of mice following a single i.p. injection is shown in Figure 13.

EXAMPLE 9

AN IN VITRO MODEL FOR EVALUATING THE EFFECT OF THE RA PEPTIDES

Materials and Methods

Cells - Fibroblasts from the inflammatory joint of an RA patient were cultured and maintained as shown in Bendersky et al, J Immunol.;188:4349-59, 2012. A quantity of 20,000 cells was added to each one of the 96 well plates with the indicated concentrations of peptide, serum amyloid A or a-lactalbumin.

WITT assay - MTT assay was effected as shown in Madhyastha et al. (2015) J Clin Diagn Res.;9 :ZC05-8.

Statistical analysis - As described in Example 2 above.

Results

An in-vitro model was developed as a tool to evaluate the biological activity of the generated RA peptides. To this end, fibroblasts from the inflammatory joint of an RA patient were incubated in-vitro and the effect of the peptides on cell survival was evaluated by a MTT assay.

As shown in Figure 14, increasing the dose of the 5-mer RA peptide (SEQ ID NO: 1) gradually inhibits cell survival. Addition 50 µg/ml Serum Amyloid A (SAA) into the fibroblast culture in combination with the 5-mer peptide prevents this inhibition. The results also indicate that a low dose of 2.5 µg/ml (~5 nM) of the peptide is able to significantly inhibit cell survival in this in-vitro model, and that the in-vitro maximal suppressive effect is 60%.

In order to demonstrate the specificity of the effect of the peptide on cell survival, the peptide was added to the cell culture at a constant concentration (25 µg/ml) and SAA
or lactalbumin (LA, a control protein with similar molecular weight) was added at escalating concentrations. As shown in Figure 15, the 5-mer peptide reduced survival of the fibroblast and addition of SAA gradually prevented this reduction in a dose response manner. In contrast, the addition of LA had no effect on the suppressive activity the peptide; indicating the SAA prevents the inhibitory effect of the 5-mer peptide in a specific manner.

In the next step, the suppressive effect of the 5-mer protected RA peptide (SEQ ID NO: 4) was compared to the 5-mer RA peptide (SEQ ID NO: 1). As shown in Figure 16, peptide modification improved the suppressive effect of the peptide.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.
WHAT IS CLAIMED IS:

1. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

2. An isolated end-capping modified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, wherein said modified polypeptide comprises an anti-inflammatory activity.

3. The end-capping modified polypeptide of claim 2, wherein said end-capping comprises an N terminus end-capping.

4. The end-capping modified polypeptide of claim 3, wherein said N terminus end-capping comprises an Acetyl.

5. The end-capping modified polypeptide of any one of claims 2-4, wherein said end-capping comprises a C terminus end-capping.

6. The end-capping modified polypeptide of claim 5, wherein said C terminus end-capping comprises an Amide.

7. The end-capping modified polypeptide of any one of claims 2-6, wherein said polypeptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

8. The isolated peptide of any one of claims 1-6, wherein said polypeptide is as set forth in SEQ ID NO: 1.

9. The end-capping modified polypeptide of claim 2 being selected from the group consisting to SEQ ID NOs: 4-6.
10. A composition of matter comprising the isolated polypeptide of any one of claims 1-9 and a non-proteinaceous moiety attached to said isolated polypeptide, wherein said isolated fusion polypeptide comprises an anti-inflammatory activity.

11. An isolated fusion polypeptide comprising the isolated polypeptide of any one of claims 1 and 8 having a C and/or N terminally attached amino acid sequence, wherein said C terminally amino acid sequence is a non-contiguous CD44vRA amino acid sequence with said isolated fusion polypeptide; and wherein said fusion polypeptide comprises an anti-inflammatory activity.

12. The composition of matter of claim 10 or the isolated polypeptide of claim 11, wherein said attached is covalent attachment.

13. The isolated polypeptide or the composition of matter of any one of claims 2-8 and 10-12, wherein said anti-inflammatory activity is not dependent on vaccination or mucosal tolerance.

14. The isolated polypeptide or the composition of matter of any one of claims 1-13, being capable of binding a protein selected from the group consisting of serum amyloid A, Transthyretin and apolipoprotein B.

15. A pharmaceutical composition comprising as an active agent the isolated polypeptide or the composition of matter of any one of claims 1-14; and a pharmaceutically acceptable carrier or diluent.

16. A method of treating an inflammatory disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated polypeptide, the composition of matter or the pharmaceutical composition of any one of claims 1-15, thereby treating the inflammatory disease in the subject.
17. Use of the isolated polypeptide, the composition of matter or the pharmaceutical composition of any one of claims 1-15, for the manufacture of a medicament for the treatment of an inflammatory disease.

18. The method of claim 16, wherein said administering comprises oral administering.

19. The pharmaceutical composition of claim 15, wherein said composition is formulated for oral administration.

20. The method of any one of claims 16 and 18 or the use of claim 17, wherein said inflammatory disease involves cells expressing CD44vRA.

21. The method of any one of claims 16 and 18 or the use of claim 17, wherein said inflammatory disease is selected from the group consisting of Rheumatoid arthritis, psoriatic arthritis, Alzheimer's disease, cancer and cardiovascular disease.

22. The method of any one of claims 16 and 18 or the use of claim 17, wherein said inflammatory disease is Rheumatoid arthritis.

23. An isolated polynucleotide comprising a nucleic acid sequence encoding the isolated polypeptide of any one of claims 1 or 11.

24. A nucleic acid construct comprising the isolated polynucleotide of claim 23.

25. A method of determining potency of a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition of any one of claims 1-15, the method comprising:

(a) contacting a batch of the isolated polypeptide, the composition of matter or the pharmaceutical composition of any one of claims 1-15 with fibroblasts obtained from an inflammatory joint of a Rheumatoid arthritis patient; and
determining survival of said fibroblasts following a predetermined incubation time, so as to determine the potency of the batch.

26. The method of claim 25, wherein said method comprising synthesizing said isolated polypeptide, said composition of matter or said pharmaceutical composition with a modification prior to said contacting.

27. The method of any one of claims 25-26, wherein reduced survival of said fibroblasts following said contacting is indicative that said batch is potent.

28. The method of any one of claims 25-27, comprising comparing said survival of said cells with survival of said cells following contacting with a reference standard batch of said isolated polypeptide, said composition of matter or said pharmaceutical composition, so as to determine the relative potency of said batch.

29. The method of any one of claims 25-28, wherein said method is effected in-vitro or ex-vivo.
FIG. 1

5-Mer peptide stability

Quantification of 100ng/ml peptide by LCMS triple Quad MRM

% stability *

~20 4 22

Temp (Celsius)

Area counts, intensity cps

Time (min)

Concentration (ng/ml)

Calibration curve

Representative pick
FIG. 2A
Injection of 25 µg RA peptide does not significantly affect paw swelling

FIG. 2B
Injection of 100 µg RA peptide does not significantly affect paw swelling

FIG. 2C
Injection of 150 µg RA peptide significantly reduces paw swelling
FIG. 13

5 mer peptide concentration in serum post single IP injection

Concentration (pg/ml)

Time (min)

0 500 1000 1500 2000 2500

15 35 60
FIG. 14

Cell survival (% of control)

Peptide (μg/ml)
INTERNATIONAL SEARCH REPORT

A61K38/00 A61K38/08 C07K14/705 C07K7/06

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 03/014160 A2 (YISSUM RES DEV CO [IL]; YAYON AVNER [IL]; NEDVETZKI SHLOMO [IL]; NAOR) 20 February 2003 (2003-02-20) claims 1-25; figures 1,8</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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

"B" earlier invention or patent but published on or after the international filing date

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

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

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

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

"X" document of particular relevance: the claimed invention cannot be considered as novel or cannot be considered to involve an inventive step when the document is taken alone

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

"A" document member of the same patent family

Date of the actual completion of the international search
16 October 2015

Date of mailing of the international search report
02/11/2015

Name and mailing address of the ISA
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Authorized officer
Volbach, Silke

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