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CYSTEINE CONTAINING PEPTIDES

(57) Abstract

Physiologically active peptides of the formula (1): A-(R₁)₅-R₂-Cys-R₃-B, wherein each variable has the meaning defined in the description, the entire peptide sequence containing up to 20 amino acid residues; and homo- or heterodimers thereof. These peptides are absorbable by the epithelial cell lining in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease.


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CYSTEINE CONTAINING PEPTIDES

Field of Invention

The present invention relates to non-antigen-specific immunomodulation, including both immunosuppression and immunostimulation. In particular, the invention relates to immunomodulatory peptides comprising cysteine amino acid residues, which peptides are capable of inducing an immunomodulatory response in mammals and thereby a therapeutic effect, and uses thereof.

Background of Invention

The immune system, when it is working properly, protects the individual from infection and from growth of cancers. In order to carry out these functions, it must be able to recognise and mount an attack against foreign antigens (including cancer-specific antigens), but not against self antigens present on normal cells throughout the body.

It is possible to stimulate the immune system in order to improve its level of protection. Vaccines, including single-protein antigens such as diphtheria toxoid, are widely used to generate immunity against a specific antigen and thus a specific disease. Where general stimulation of the immune system is desired, this can sometimes be achieved with nonspecific agents such as adjuvants, interleukins, interferons, and colony stimulating factors.

Occasionally, the immune system loses its critical ability to distinguish self from non-self. The resulting immunological assault on the individual’s own tissues can take the form of autoimmune disease: for example, systemic lupus erythematosus, Type 1 diabetes, or rheumatoid arthritis. In such a case, or alternatively where the individual is the recipient of a transplanted organ or tissue, suppression rather than stimulation of the immune response is desirable.
Non-specific down-regulation of the immune response is typically achieved by treatment with corticosteroids, azathioprine, cyclosporine, tacrolimus (FK506), rapamycin, or mycophenolate mofetil. Certain immunoglobulins, including the monoclonal antibody OKT3, have also been used for this purpose. Suppression of immunity against a specific antigen, called “tolerance induction”, may also be possible. Methods that have been used for inducing tolerance against a particular antigen include intravenous or repeated topical administration of the antigen in dilute form, treatment with a very high dose of the antigen, and oral administration of the antigen.

It has now been found that certain peptides containing cysteine amino acid residues have activity as immunomodulators. A surprising property associated with the immunomodulatory activity is that the activity has been found to be immunoinhibitory or immunostimulatory in effect, on the basis of experiments described herein and furthermore, that the immunomodulatory activity has been shown to be indicative of having some therapeutic effect in the treatment of certain diseases, such as cancer and arthritis.

Furthermore, it has been found that when the administration to the epithelial cell lining is by way of oral administration, the administration of the peptides has been observed to correlate with a modulating effect on the growth of tumours.

A further surprising finding is that the oral presentation of “naked” peptides of the invention did not require the inclusion of added transport agents. Thus, the peptides of the invention do not need to be administered in association with transport agents such as delivery vehicles e.g. vesicular delivery systems which are designed to improve delivery to the mucosal epithelial cell lining of the gut.
In addition, it has been found that the amount of peptide required to produce the therapeutic effect by oral delivery can be significantly lower than that required to produce a similar effect when the peptide is delivered systemically, eg by parenteral injection.

It is an object of the present invention to provide an effective means for treating disease using immunomodulatory peptides.

It is another object of the invention to provide immunomodulatory peptides which can be utilised in the treatment of disease.

These and other objects of the invention will become apparent from the following description and examples.

Prior Art

WO96/11943 describes peptides with immunomodulatory effect comprising 4 to 15 amino acids and represented by the general formula A-X-Y-Cys-Z-B (wherein X is Gly, Ala, Ile, Asp, Thr, Ser, Arg or Trp, Y is Pro, pipercolic acid or Ile and Z is selected from Ile, Phe, Pro, Ala, Tyr or Gly). Dimers of these monomers formed by intramolecular disulphide bonds resulting from oxidization of the cysteine residues are described. Also disulphides linked cyclised monomers are described.

WO92/14834 purports to describe to insulin-like growth factor binding protein isolated from rat serum. Cysteine-residue-containing peptides of 25 amino acid residues or longer are identified.

US-A-5510332 purports to describe peptides which inhibit the binding of α₄β₁ integrin to a protein such as VCAM-1 or fibronectin. Sequence 62 has, at the N-terminal, Gly Pro Cys Trp.
WO98/05783 purports to describe novel peptide sequences useful for vaccinating cats against feline leukemia virus and humans against human retroviruses. Amongst the many peptides set out, some have sequences terminating in the amino acids GLC, LIC, GIC.

JP-A2-08151396 purports to describe HLA-oligopeptides and immunomodulatory agents containing them. Numerous oligopeptide sequences are disclosed, some of which are cysteine-containing.

US 5453272 purports to describe a lectin derived carbohydrate binding peptide which inhibits cell-mediated immune responses and having the amino acid sequence SPYGRC.

WO92/00995 and WO94/15958 purport to describe cyclic short-chain peptides useful in modulating cell adhesion. The cyclising moiety may be a disulphide bridge between cysteine residues.

WO96/06108 purports to describe cyclic peptides that inhibit the binding between VLA-4 receptor expressed on inflammatory leukocytes and the fibronectin CS-1 peptide expressed on endothelial cells. There is mentioned a cyclised peptide of the formula:

\[ R{-}Xaa{-}Z_1{-}Asp{-}Phe{-}Y_2{-}Xaa{-}NH_2 \]

with at least one Xaa being oxidized cysteine and the other being oxidized cysteine, homocysteine, or penicillamine such that the two Xaa's together form a disulphide bond. \( Y_2 \) may be absent, Pro, Pro-Ser or Pro-Ser-Thr.

WO96/22106 purports to describe compounds which inhibit CD8 mediated T-cell activation and have a molecular surface that corresponds to the molecular surface of human CD at specified amino acids. Among conformationally restricted peptides described, decapeptides including \(-R_{28}R_{29}R_{30}\), wherein \( R_{28} \) is glutamine or asparagine, \( R_{29} \) is arginine and \( R_{30} \) is cysteine or penicillamine are mentioned.
EP-A-0425212 purports to describe cyclic anti-aggregatory short-chain peptides. The cyclising moiety may be a disulphide bridge. The linking bridges are generally formed through cysteine residues.

WO95/11917 and WO96/17861 purport to describe substituted tetra- and penta-peptide inhibitors of protein farnesyl transferase useful for controlling tissue proliferative diseases. Many tetra- and penta-peptides are described some of which are cysteine-containing.


EP-A-0603399 purports to describe novel peptides isolated from the product culture of a streptomyces and having an endothelin antagonism and an activity of suppressing the rise of an intracellular calcium or cyclic guanosine-3,5-phosphate concentration caused by endothelin. Sequences 3 and 4 contain the amino acid residues Ala-Pro-Cys-Trp (SEQ. ID. NO. 1).


Statement of Invention

According to one aspect of the present invention, there are provided physiologically active peptides of formula (I):

\[ A^\left(R_1\right)_{x}^R_2-Cys-R_3-B \]  

(I)

wherein
each A is independently selected from H, a protecting group e.g. ethyl, trityl (Trt), allyl, or t-butyl, or at least one amino acid residue independently selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

x is 0 (R₁ being absent), and

R₂ and R₃ are residues of amino acids chosen from one of the following combinations (a) and (b) of amino acids

(a) R₂ leucine (Leu), R₃ phenylalanine (Phe)
(b) R₂ glutamine (Gln), R₃ alanine (Ala), or

x is 1, and

R₁ and R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(d) R₁ glycine (Gly), R₂ proline (Pro), R₃ methionine (Met)
(e) R₁ alanine (Ala), R₂ proline (Pro), R₃ tryptophan (Trp)
(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(m) R₁ alanine (Ala), R₂ leucine (Leu), R₃ arginine (Arg)
(n) $R_1$ glycine (Gly), $R_2$ alanine (Ala), $R_3$ proline (Pro)
(o) $R_1$ lysine (Lys), $R_2$ serine (Ser), $R_3$ lysine (Lys)
(p) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ phenylalanine (Phe)
(q) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ tyrosine (Tyr)
(r) $R_1$ serine (Ser), $R_2$ proline (Pro), $R_3$ methionine (Met)
(s) $R_1$ glycine (Gly), $R_2$ proline (Pro), $R_3$ tryptophan (Trp),

with the provisos that, when $x$ is 0 ($R_1$ is absent), $A$ is H or a protecting group, and, when $R_1$ is glycine (Gly), $A$ is said at least one amino acid residue, and

each B is independently selected from the group consisting of OH, NH$_2$, an oxygen or a nitrogen carrying a protecting group, such as ethyl, trityl (Trt), allyl or t-butyl, or at least one amino acid residue selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

the entire peptide sequence containing up to 20 amino acid residues; and

homo- or heterodimers thereof for use in therapy as immunomodulatory agents.

According to another aspect of the present invention, there are provided, as novel compounds, peptides of formula (I)

wherein $x$ is 0 ($R_1$ being absent), and

$R_2$ and $R_3$ are residues of amino acids chosen from one of the following combinations (a) and (b) of amino acids

(a) $R_2$ leucine (Leu), $R_3$ phenylalanine (Phe)
(b) $R_2$ glutamine (Gln), $R_3$ alanine (Ala), or
x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations

(c) and (f) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(m) R₁ alanine (Ala), R₂ leucine (Leu), R₃ arginine (Arg)
(n) R₁ glycine (Gly), R₂ alanine (Ala), R₃ proline (Pro)
(o) R₁ lysine (Lys), R₂ serine (Ser), R₃ lysine (Lys)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r) R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),

with the provisos that, when x is 0 (R₁ is absent), A is H or a protecting group, and, when R₁ is glycine (Gly), A is said at least one amino acid residue,

and homo- or heterodimers thereof.

Amino acid residues of A and B independently selected from amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, and sulphur containing side chains. Suitable
amino acids may be independently selected from the groups comprising naturally and non-naturally occurring amino acid residues. Examples of naturally occurring amino acid residues include isoleucine (Ile), leucine (Leu), alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), lysine (Lys), phenyl alanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), methionine (Met), valine (Val) and histidine (His). Naturally, the skilled addressee will appreciate that naturally occurring amino acid residues means those amino acid residues which are found in peptides and/or proteins of living organisms. The skilled addressee will also appreciate that such naturally occurring amino acid residues may be present in peptides of the invention in chemically modified forms e.g. including added protecting groups such as ethyl, trityl (Trt), allyl, t-butyl and the like. Naturally, the skilled addressee will appreciate that any protecting group(s) which may be present on the peptides of the invention should be such so as not to substantially interfere with the immunomodulatory properties thereof and hence therapeutic effect thereof.

Cysteine residues within formula (I) are capable of reacting to form intermolecular links with the formation of dimers or intramolecular bridges with the formation of cyclised monomers.

The amino acid units making up the peptides may be in L- and D-form. Generally the amino acid units will be in the L-form.

Peptides of the invention can be made synthetically, for example by chemical means, or through the use of recombinant DNA technology. Alternatively, peptides according to the invention can be isolated from polypeptides or proteins or the like.

A peptide of the invention may take the form of a dimer consisting of two like or two dissimilar peptide monomers linked to each other by way of covalent bonds (e.g. sulphur to sulphur bonds). In an aspect of the invention, the peptide monomers may be linked one to the other through bonds between at least a cysteine amino acid residue of the first
monomer and at least one of cysteine amino acid residue of a second monomer. Dimers of the present invention may be in parallel form, i.e. two peptide monomers aligned parallel one to the other such that both the peptide monomers are readable in one direction eg from the N-terminal to C-terminal direction. The peptide monomers making up the dimer may or may not be the same length. Preferably, the peptide monomers are the same length and the N- and C-terminal amino acid residues of one monomer are located adjacent to the N- and C-terminal amino acid residues of the second peptide monomer. Alternatively, a dimer of the present invention may be in anti-parallel form. That is to say, a first monomer read from the N-terminal amino acid residue to the C-terminal amino acid residue is aligned against a second monomer which is read from the C-terminal amino acid residue to the N-terminal amino acid residue ie in the opposite direction to that of the first peptide monomer; the two peptide monomers being linked through covalent bonds as described above for parallel or anti-parallel dimers of the invention.

Where the two peptide monomers forming a peptide dimer of the invention are dissimilar one to the other, the dimer is referred to as an heterodimer. A heterodimer can be in parallel or anti-parallel form. Thus, a heterodimer can be composed of two peptide monomers of the same length, differing, for example, in the substitution of an amino acid residue having the L- form to an amino acid residue having the D- form. Alternatively, the lengths of the peptide monomers making up the dimer may be different. In a heterodimer, at least one of the monomers is of formula (I) and generally both are of formula (I).

Preferably, the first and second peptide monomers making up a dimer of the present invention are the same.

Peptides of the invention also include monomers which can be linear or cyclic e.g. wherein at least two cysteine amino acid residues are linked through disulphide bridges. Preferably, the monomers are linear.
Administration of peptides of the invention by way of, for example, oral administration, intra-tracheal, nasal or parenteral administration gives rise to a measurable modulated immune response, as indicated in the examples herein.

"Epithelial cell lining" is defined as being the cell lining and associated cells thereto which covers the internal and external surfaces of the body, including the lining of vessels and other small cavities. For the purposes of the present invention the epithelial cell lining is regarded as being at least one cell layer in depth and as many as several cell layers deep. Cells included within the ambit of "epithelial cell lining" also includes those cells and specialised lymphoid tissues which are located in or associated with the said epithelial cell lining and which influence the immune response such as T-lymphocytes, B-lymphocytes, enterocytes, NK-cells, monocytes, dendritic cells and cells comprising mucosal associated lymphoid tissue (MALT), such as Peyer’s patches and the like. Thus, the skilled addressee will appreciate that so-called migratory cells, such as T- and B-lymphocytes which can be regarded as transient resident cells of the epithelial cell lining as defined above are included within the ambit of the definition of epithelial cell lining. The peptides of the invention may be absorbed by the epithelial cell lining in a passive or active sense. For example, the peptides may be absorbed on the cell surface, or actively or passively taken up by cells located on the lumen surface side of the epithelial cell lining, or they may pass in between cells located on the lumen surface side of the epithelial cell lining and are taken up by cells located deeper in the epithelial cell lining eg T-lymphocytes or Peyer’s patches. The skilled addressee will also appreciate that "absorption" as defined herein also includes the situation wherein peptides of the invention initiate an immune response by interacting with cell surface receptors found in or on the membranes of certain specialised cells located in the epithelial cell lining, such as on enterocytes, and intra-epithelial lymphocytes, without physically penetrating the epithelial cell lining. Thus, the skilled addressee will understand that peptides of the invention may interact with, bind to, pass through or penetrate the epithelial cell lining.
The peptides of the invention are preferably administered by oral, nasal, or intra-tracheal administration in oral, nasal or intra-tracheal dosage forms. It has been found that the amount of a peptide of the invention required to produce a given therapeutic effect when orally administered can be significantly lower than that required to produce the same effect via other types of administration, such as parenteral administration.

In a further aspect of the invention there is provided an oral dosage form comprising at least one immunomodulatory peptide according to the invention, the at least one peptide being absorbable by the epithelial cell lining of the gastrointestinal tract in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease.

In a further aspect of the invention there is provided an oral dosage form comprising at least one immunomodulatory peptide according to the invention, the at least one peptide being absorbable by the epithelial cell lining of the gastrointestinal tract in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease wherein the amount of the at least one orally administered peptide needed to induce an observable level of modulated immune response in a mammal is less than the amount of the same at least one peptide administered parenterally and needed to achieve a similar observable level of modulated immune response in the said mammal.

In a further aspect of the invention there is provided a nasal dosage form comprising at least one immunomodulatory peptide according to the invention, the at least one peptide being absorbable by the epithelial cell lining of the nasal passages in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease.

In a further aspect of the invention there is provided a nasal dosage form comprising at least one immunomodulatory peptide according to the invention, the peptide being absorbable by the epithelial cell lining of the nasal passages in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease wherein the amount of the nasally administered peptide needed to induce an observable level of
modulated immune response in a mammal is less than the amount of the same peptide administered parenterally and needed to achieve a similar observable level of modulated immune response in the said mammal.

In a further aspect of the invention there is provided an intra-tracheal dosage form comprising at least one immunomodulatory peptide according to the invention, the at least one peptide being absorbable by the epithelial cell lining of the lung in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease.

In a further aspect of the invention there is provided an intra-tracheal dosage form comprising at least one immunomodulatory peptide according to the invention, the at least one peptide being absorbable by the epithelial cell lining of the lung in a mammal resulting in a modulated immune response and thereby a therapeutic effect against disease wherein the amount of the at least one intra-tracheally administered peptide needed to induce an observable level of modulated immune response in a mammal is less than the amount of the same at least one peptide administered parenterally and needed to achieve a similar observable level of modulated immune response in the said mammal.

Peptides of the invention contain up to 20 amino acid residues in the or each sequence of formula (I). Preferably, the or each peptide sequence is from 4 amino acid residues to 20 amino acid residues in length. More preferably, the or each peptide sequence is from 4 to 15 amino acids in length (e.g. 4 to 10 or 4 to 9), and, most preferably, from 4 to 7 amino acids in length. For example, the peptide sequences can be 4, 5, 6, 7, or 8 amino acid residues in length, with or without protecting groups.

The peptides of the invention may or may not be associated with transport agents as defined herein. Preferably, the peptides of the invention are administered in a "naked" form ie free from added transport agents. Added transport agents are those with which the peptides of the invention are intentionally placed in contact or in association either before,
during or immediately after administration and which may serve to improve absorption and/or improve the stability of the peptide.

Thus, in one preferment there is provided a physiologically active peptide according to the invention free from added transport agents.

Within formula (I), \( R_1 \) to \( R_3 \) are residues of the amino acids selected from the following:

\[
\begin{align*}
R_1 &= E & R_2 &= P & R_3 &= M \\
R_1 &= G & R_2 &= P & R_3 &= M \\
R_1 &= A & R_2 &= P & R_3 &= W \\
R_1 &= A & R_2 &= P & R_3 &= M \\
R_1 &= E & R_2 &= P & R_3 &= W \\
R_1 &= S & R_2 &= P & R_3 &= W \\
R_1 &= L & R_2 &= L & R_3 &= G \\
R_1 &= \text{absent} & R_2 &= L & R_3 &= F \\
R_1 &= P & R_2 &= R & R_3 &= R \\
R_1 &= G & R_2 &= Y & R_3 &= P \\
R_1 &= V & R_2 &= V & R_3 &= N \\
R_1 &= A & R_2 &= L & R_3 &= R \\
R_1 &= G & R_2 &= A & R_3 &= P \\
R_1 &= K & R_2 &= S & R_3 &= K \\
R_1 &= E & R_2 &= P & R_3 &= F \\
R_1 &= E & R_2 &= P & R_3 &= Y \\
R_1 &= S & R_2 &= P & R_3 &= M \\
R_1 &= G & R_2 &= P & R_3 &= W \\
R_1 &= \text{absent} & R_2 &= Q & R_3 &= A
\end{align*}
\]

For example:

\[ R_1 = E \quad R_2 = P \quad R_3 = M \]
R1=G  R2=P  R3=M
R1=A  R2=P  R3=W
R1=A  R2=P  R3=M
R1=E  R2=P  R3=W
R1=S  R2=P  R3=W
R1=S  R2=P  R3=M
R1=G  R2=P  R3=W
R1=E  R2=P  R3=F
R1=E  R2=P  R3=Y

or:
R1=P  R2=R  R3=R
R1=G  R2=Y  R3=P
R1=V  R2=V  R3=N
R1=A  R2=L  R3=R
R1=G  R2=A  R3=P
R1=K  R2=S  R3=K
R1=absent  R2=Q  R3=A

Suitably:
R1=L  R2=L  R3=G
R1=absent  R2=L  R3=F

In the above, the peptides are represented by the standard 1-letter code. Also, of course R1 corresponds to R₁ of formula (I), R₂ to R₂ and R₃ to R₃. R₁=absent corresponds to the case when x = 0.

Preferred peptides for use according to the invention are those wherein

x is 0 (R₁ being absent), and
$R_2$ and $R_3$ are residues of amino acids of the following combination (b) of amino acids

(b) $R_2$ glutamine (Gln), $R_3$ alanine (Ala), or

$x$ is 1, and

$R_1$, $R_2$ and $R_3$ are residues of amino acids chosen from one of the following combinations (c) to (l) and (p) to (s) of amino acids

(c) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ methionine (Met)

d) $R_1$ glycine (Gly), $R_2$ proline (Pro), $R_3$ methionine (Met)

e) $R_1$ alanine (Ala), $R$ proline (Pro), $R_3$ tryptophan (Trp)

(f) $R_1$ alanine (Ala), $R_2$ proline (Pro), $R_3$ methionine (Met)

g) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ tryptophan (Trp)

(h) $R_1$ serine (Ser), $R_2$ proline (Pro), $R_3$ tryptophan (Trp)

(i) $R_1$ leucine (Leu), $R_2$ leucine (Leu), $R_3$ glycine (Gly)

(j) $R_1$ proline (Pro), $R_2$ arginine (Arg), $R_3$ arginine (Arg)

(k) $R_1$ glycine (Gly), $R_2$ tyrosine (Tyr), $R_3$ proline (Pro)

(l) $R_1$ valine (Val), $R_2$ valine (Val), $R_3$ asparagine (Asn)

(p) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ phenylalanine (Phe)

(q) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ tyrosine (Tyr)

(r) $R_1$ serine (Ser), $R_2$ proline (Pro), $R_3$ methionine (Met)

(s) $R_1$ glycine (Gly), $R_2$ proline (Pro), $R_3$ tryptophan (Trp),

with the provisos that, when $x$ is 0 ($R_1$ is absent), $A$ is H or a protecting group, and, when $R_1$ is glycine (Gly), $A$ is said at least one amino acid residue.

Preferred novel peptides according to the invention are those wherein
x is 0 (R₁ being absent), and

R₂ and R₃ are residues of amino acids of the following combination (b) of amino acids

(b) R₂ glutamine (Gln), R₃ alanine (Ala), or

x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations

(c) to (l) and (p) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r) R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),

with the provisos that, when x is 0 (R₁ is absent), A is H or a protecting group, and, when
R₁ is glycine (Gly), A is said at least one amino acid residue.

More preferred peptides according to the invention include those wherein

x is 1, and
R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c), (g), (h), (j) to (l), (p), (q) and (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tyrosine (Tyr),

with the proviso that, when R₁ is glycine (Gly), A is said at least one amino acid residue.

Particularly preferred such peptides include those wherein

x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c) and (j) to (l) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn),

with the proviso that, when R₁ is glycine (Gly), A is said at least one amino acid residue.
Preferred peptides include those of the formulae:

Leu Ala Phe Glu Pro Cys Met \hfill (SEQ. ID. NO. 3)
Val Leu Trp Glu Pro Cys Trp \hfill (SEQ. ID. NO. 4)
Met Leu Phe Ser Pro Cys Trp \hfill (SEQ. ID. NO. 5)
(Met Leu Phe Ser Pro Cys Trp)\textsubscript{2} \hfill (SEQ. ID. NO. 5)
(Ala Val Pro Glu Pro Cys Phe)\textsubscript{2} \hfill (SEQ. ID. NO. 6)
(Met Met Tyr Glu Pro Cys Tyr)\textsubscript{2} \hfill (SEQ. ID. NO. 7)
(Val Ala Tyr Gly Pro Cys Trp)\textsubscript{2} \hfill (SEQ. ID. NO. 8)
Leu Arg Pro Arg Cys Arg Pro \hfill (SEQ. ID. NO. 9)
Pro Gln Val Val Cys Asn Tyr \hfill (SEQ. ID. NO. 10)
Arg Gly Tyr Cys Pro Tyr \hfill (SEQ. ID. NO. 11)

Within formula (I) are peptides wherein

R\textsubscript{1} is a residue of an amino acid selected from serine (Ser), alanine (Ala), and glycine (Gly),

R\textsubscript{2} is a proline (Pro) residue, and

R\textsubscript{3} is a residue of an amino acid selected from methionine (Met), and tryptophan (Trp),

provided that when R\textsubscript{1} is glycine, A is said at least one amino acid residue, and

R\textsubscript{1} is a glutamic acid (Glu) residue,

R\textsubscript{2} is a proline (Pro) residue,

R\textsubscript{3} is a residue of an amino acid selected from methionine (Met), tryptophan (Trp),

phenylalanine (Phe), or tyrosine (Tyr).
Novel such peptides include those wherein

R₁ is a serine (Ser) residue,

R₂ is a proline (Pro) residue, and

R₃ is a residue of an amino acid selected from methionine (Met), and tryptophan (Trp), and

R₁ is a glutamic acid (Glu) residue,

R₂ is a proline (Pro) residue,

R₃ is a residue of an amino acid selected from methionine (Met), tryptophan (Trp), phenylalanine (Phe), or tyrosine (Tyr).

When A and/or B represent an amino acid residue or a sequence of amino acid residues, the amino acid residue or sequence of amino acid residues can include naturally occurring amino acid residues, such as those described hereinabove or analogues thereof or can include non-naturally occurring amino acid residues, such as synthetic amino acid residues and analogues thereof, or amino acid residues or sequences of amino acid residues including both naturally occurring amino acid residues and/or analogues thereof and non-naturally occurring amino acid residues and/or analogues thereof.

The skilled addressee will also appreciate that included within the scope of formula (I) are peptides in which intramolecular, e.g. disulphide, bridges are present between contiguously aligned or spaced apart, cysteine residues. Such peptides represent an oxidised form of peptides of formula (I).

The skilled addressee will also appreciate that the peptides of formula (I) can be in the form of homodimers or heterodimers which may be in parallel or anti-parallel form. Such
homodimers or heterodimers are comprised of monomers of formula (I) and can be linked through covalent, e.g. sulphur to sulphur bonds, between a cysteine residue of one monomer and a cysteine residue of another monomer.

Also included within the ambit of the invention are pharmaceutically acceptable salts of peptides of formula (I) or physiologically functional derivatives thereof together with a pharmaceutically acceptable carrier therefor.

The skilled addressee will further appreciate that the peptides of the invention will include within their ambit variants of the formula (I) which contain one or more modifications of the peptide backbone and which retain the immunomodulatory properties according to the invention. Such modifications have been reviewed for example by A.F. Spatola “Chemistry and Biochemistry of Amino Acids, Peptides and Proteins”; B. Weinstein, Ed; Marcel Dekker, New York, 1983, Vol 1, Chapter 5; Robert A Wiley et al, “Peptidomimetics derived from natural products” Medicinal Research Reviews, Vol 13, No. 3, 327-384 (1993) John Wiley & Sons Inc; and Youe Kong Shue et al, “Double Bond Isoteres of the Peptide Bond” Bioorganic and Medicinal Chemistry, Vol 1, No. 3, 161-171 (1993) Pergamon Press Ltd.

The peptides of the invention can be administered with or without transport agents. Preferably, peptides of the invention are administered orally, intra-tracheally, nasally, or systemically free from added transport agents. More preferably, the peptides of the invention are administered intra-tracheally, nasally, or orally. Most preferably, the peptides of the invention are administered orally. “Transport agents” includes added means for delivery such as vesicular delivery systems, micro particles, liposomes, and like systems which are designed to carry drugs (e.g peptides) to the epithelial cell lining or endothelial cell lining. “Transport agents” also includes chemicals or additional peptide sequences which may form an association with, or are fused to, or are complexed with the peptides and which help to maintain physiological integrity of peptide sequences of the invention, for example, presenting the peptides in a prepro- or pro-form or fusing the peptides to
carrier proteins, eg glucosyl transferase, or complexed to chemical agents, such as cyclodextrins and the like. Preferably, peptides of the invention are administered to the recipient as free peptides along with the usual adjuvants, excipients and diluents commonly found in pharmaceutical formulations. Thus, peptides of the invention can be delivered by oral or systemic administration in simple oral or systemic formulations comprising adjuvants, diluents and excipients commonly employed in oral and systemic dosage forms. Preferably, the peptides are administered in an oral dosage form free from added transport agents.

Mucosal associated lymphoid tissue (MALT) is also found in the epithelial cell linings of the gastrointestinal tract, ie, oesophagus, stomach, duodenum, ileum, and colon; bronchiolar linings in the lung; and in the linings of the nasal passages. Without the intention of being bound by theory, it is thought that the peptides of the invention interact with MALT and thereby set in train a sequence of immunomodulating events which results in a therapeutic effect against certain diseases.

The immunomodulatory response can be immunoinhibitory or immunostimulatory in effect. The immunomodulatory response has been shown to be indicative for therapy against cancer. The peptides of the invention having an immunomodulatory effect are indicated as being advantageous in the treatment of cancers of mesenchymal origin such as sarcoma, eg, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma or chordosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, synoviosarcoma or mesotheliosarcoma; leukemias and lymphomas such as granulocytic leukemia, monocytic leukemia, lymphocytic leukemia, malignant lymphoma, plasmocytoma, reticulum cell sarcoma or Hodgkins disease; sarcomas like leiomyosarcoma or rhabdomyosarcoma, tumours of epithelial origin (Carcinomas) such as squamous cell carcinoma, basal cell carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, adenocarcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, undifferentiated carcinoma, bronchogenic carcinoma, melanoma, renal cell carcinoma, hepatoma-liver cell carcinoma, bile duct carcinoma,
cholangiocarcinoma, papillary carcinoma, transitional cell carcinoma choriocarcinoma, seminoma or embryonal carcinoma; and tumours of the central nervous system like glioma, meningoma, medulloblastoma, schwannoma or ependymoma. Peptides of the invention are indicated on the basis of their activity for the treatment of malignancies such as melanoma, mammary carcinoma, gastrointestinal carcinoma such as colonic carcinomas, glioma, bladder carcinoma and squamous cell carcinoma of the neck and head region. Furthermore, peptides according to the invention are indicated for therapy in the treatment of acute and/or chronic infections associated with autoimmune disease and autoimmune disease per se such as non-obese diabetes, systemic lupus erythematosus, scleroderma, Sjögren's syndrome, dermatomyositis or multiple sclerosis, rheumatoid arthritis, artherosclerosis, and psoriasis, asthma, rhinitis, fibrosis, chronic bronchitis, hepatitis, post-infectious anergy, acquired immune deficiency diseases such as AIDS, HIV and post traumatic immunological anergy.

The peptides according to the present invention may, if appropriate, be used together with a traditional therapy regime, such as with methotrexate (MTX).

Moreover, the peptides according to the present invention, being immunomodulatory in action, may be advantageously employed as adjuvants in various forms of vaccine preparations and in formulations designed to inhibit rejection of organs in transplants.

In another aspect of the invention, there is provided a method of inducing a modulated immune response in a mammal which comprises administering to the epithelial cell lining of the mammal a dose of a peptide according to the invention, enough to induce said modulated immune response and thereby a therapeutic effect.

In a further aspect of the invention there is provided a method of inducing a modulated immune response in a mammal which comprises 1) identifying a mammal in need of modulation of its immune response and 2) administering to at least one epithelial cell lining of the mammal a dose of a peptide according to the invention, enough to induce said
immunomodulatory response and thereby a therapeutic effect. Preferably, the epithelial cell lining to which the peptide is administered is the epithelial cell lining of the gastrointestinal tract. Most preferably, the peptide is administered to the MALT.

In a preferment there is provided a method of inducing a modulated immune response in a mammal which comprises administering to MALT of the mammal a dose of a peptide according to the invention, said peptide being free from added transport agents and being sufficient to induce said modulated immune response and thereby a therapeutic effect.

In a further aspect of the invention there is provided use of a peptide according to the invention, in the preparation of a medicament suitable for the treatment of disease. Particular forms of cancer which may be treated with peptides of the invention are listed hereinabove.

The peptides may be used in combination with surgery pre-, or more preferably, post-operationally.

In a preferment, there is provided use of a peptide according to the invention free from added transport agents in the preparation of a medicament suitable for the treatment of disease, in particular cancer and rheumatoid arthritis.

In a further embodiment of the invention there is provided a method of making a peptide of the invention by a chemical process in which individual amino acid residues or fragments of peptides of the invention are joined to form peptide bonds and wherein protecting groups are employed at the beginning and/or end of the process.

In another embodiment of the invention there is provided as a further alternative aspect of the invention a physiologically active peptide according to the invention, preferably free from added transport agents, for use in therapy, for example, in cancer or rheumatoid
arthritus therapy. In a preferment, there is provided a peptide of the invention for use in
therapy, for example in cancer therapy or rheumatoid arthritis therapy.

The amount of a peptide according to the invention which is required in cancer or
rheumatoid arthritis therapy will, of course, vary and is ultimately at the discretion of the
medical or veterinary practitioner. The factors to be considered include the condition being
treated, the route of administration, and nature of the formulation, the mammal’s body
weight, surface area, age, and general condition and the particular peptide to be
administered. A suitable effective dose of peptides of the invention generally lies in the
range of from about 0.0001 μmol/kg to about 1000μmol/kg bodyweight, preferably from
about 0.003 to about 300 μmol/kg body weight, e.g. in the range of from about 0.001 to
100 μmol/kg bodyweight, for example, 0.03 to 3.0 μmol/kg bodyweight. The total dose
may be given as a single dose or multiple doses, e.g two to six times per day. For example,
for a 75 kg mammal (e.g. a human) the dose range would be about 2.25 μmol/kg/day to
225 μmol/kg/day and a typical dose could be about 100 μmol of peptide. If discrete
multiple doses are indicated treatment might typically be 25 μmol of a peptide of the
invention given up to 4 times per day. In an alternative administrative regimen, peptides of
the invention may be given on alternate days or even once or twice a week. The skilled
addressee will appreciate that an appropriate administrative regimen would be at the
discretion of the physician or veterinary practitioner.

Whilst it is possible for the active peptide to be administered alone, it may be preferable to
present the active peptide in a pharmaceutical formulation. Formulations of the present
invention, for medical use, comprise a peptide of the invention or a salt thereof together
with one or more pharmaceutically acceptable carriers and optionally other therapeutic
ingredients. The carrier(s) should be pharmaceutically acceptable in the sense of being
compatible with the other ingredients of the formulation and substantially non-deleterious
to the recipient thereof. The skilled addressee will appreciate that free acid addition salts
(e.g. hydro-halo salts) of peptides referred to herein as well as base salts are encompassed
within the ambit of the invention. Most preferably the salts will be pharmaceutically acceptable.

Suitable acid addition salts include those formed from hydrochloric, hydrobromic, nitric, perchloric, sulphuric, citric, tartaric, phosphoric, lactic, benzoic, glutamic, oxalic, aspartic, pyruvic, acetic, succinic, fumaric, maleic, oxaloacetic, isethionic, stearic, phthalic, methanesulphonic, p-toluene sulphonic, benzenesulphonic, lactobionic, glucuronic and trifluoracetic acids. Suitable base salts include inorganic base salts such as alkali metal (e.g. sodium and potassium) salts and alkaline earth metal (e.g. calcium) salts; organic base salts e.g. phenylethylbenzylamine, dibenzylethylenediamine, ethanolamine and diethanolamine salts; and amino acid salts e.g. lysine and arginine. Most preferably, the salts will be pharmaceutically acceptable.

The present invention, therefore, further provides a pharmaceutical formulation comprising a peptide of the invention together with a pharmaceutically acceptable carrier therefor.

Naturally, the skilled addressee will appreciate that any pharmaceutical formulation comprising a peptide of the invention can include more than one peptide of the invention. Thus, a pharmaceutical formulation may comprise at least two peptides of the invention or a cocktail of peptides of the invention.

There is also provided a method for the preparation of a pharmaceutical formulation comprising bringing into association one or more peptides of the invention, or a physiologically functional derivative thereof, and a pharmaceutically acceptable carrier therefor.

The peptides of the invention and physiologically functional derivatives thereof may be administered by any route appropriate to the condition to be treated, suitable routes including oral, intra-tracheal, rectal, nasal, topical (including buccal and sublingual), vaginal, and parenteral (including subcutaneous, intramuscular, intravenous, intradermal,
intrathecal, intraperitoneal, and epidural). It will be appreciated that the route may vary with, for example, the condition of the recipient. Preferred formulations are those suitable for oral, nasal or intra-tracheal administration. Most preferred formulations are those suitable for oral administration.

Formulations for topical administration in the mouth include lozenges comprising the peptide(s) in a flavoured basis, usually sucrose and acacia and tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouth washes comprising the peptide(s) in a suitable liquid carrier.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets, lozenges comprising the peptide(s) in a flavoured base, usually sucrose and acacia and tragacanth; pastilles comprising the active ingredient(s) in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouth washes comprising the active ingredient(s) in a suitable liquid carrier. Each formulation generally contains a predetermined amount of the active peptide(s); as a powder or granules; or a solution or suspension in an aqueous or non-aqueous liquid such as a syrup, an elixir, an emulsion or draught and the like.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active peptide(s) in a free-flowing form such as a powder or granules, optionally mixed with a binder, (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered peptide(s) moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.
A syrup may be made by adding the active peptide(s) to a concentrated, aqueous solution of a sugar, for example, sucrose, to which may also be added any necessary ingredients. Such accessory ingredient(s) may include flavourings, an agent to retard crystallisation of the sugar or an agent to increase the solubility of any other ingredients, such as a polyhydric alcohol, for example, glycerol or sorbitol.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives, (including antioxidants) and the like.

Emulgents and emulsion stabilisers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulphate.

The choice of suitable oils or fats for the formulation is based on achieving the desired therapeutic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate, or a blend of branch-chained esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations for rectal administration may be presented in any suitable form e.g. as a suppository with a suitable base comprising peptide(s) of the invention in admixture with a
neutral fatty base, for example cocoa butter, or, for example in admixture with a salicylate, 
or in the form of solutions and suspensions. In an alternative, formulations in the form of 
gelatin rectal capsules comprising active peptide(s) of the invention in admixture with 
vegetable oil(s) or paraffin oil can be used.

Formulations suitable for nasal administration wherein the carrier is a solid include a 
coarse powder having a particle size for example in the range 20 to 500 microns. Where the 
particle size relates to an active substance in particle form per se, the particle size may be 
in the range of from 2 to 500 microns. Coarse powder formulations can be administered by 
rapid inhalation through the nasal passage from a container of the powder held up close to 
the nose. Suitable formulations wherein the carrier is a liquid, for administration as for 
example a nasal spray or as nasal drops, include aqueous or oily solutions of the active 
ingredient. Thus, peptides of the invention may be formulated in pressurised metered dose 
inhalers or dry powder inhalers for oral or nasal inhalation or in liquid formulations for 
nebulisation. The active peptide(s) is micronised or otherwise processed to a particle size 
suitable for inhalation therapy (mass median diameter < 10 μm).

In the case of pressurised metered dose inhalers the micronised peptide(s) can be 
suspended in a liquefied propellant or a mixture of liquefied propellants. Such propellants 
can also, but not necessarily act as solvents. In either case, the micronised peptide(s) can be 
filled into a container equipped, for example with a metering valve.

Suitable propellants include those commonly employed in the art, such as, 
hydrofluoroalkanes (HFAs). The HFA propellants can be present in any mixture which is 
appropriate for delivering peptide(s) of the invention to MALT. Examples of suitable 
HFAs for use in the invention include tetrafluoroethane (eg propellant 134a (Hoechst)) and 
heptafluoropropane (eg propellant 227 (Hoechst)). Naturally, the skilled addressee will 
appreciate that appropriate concentrations of surfactants can also be present in such 
formulations, for example, sorbitan trioleate, lecithin, oleic acid and the like, the use of 
surfactants being to increase the physical stability of the peptide(s) preparation. The
formulation can also contain solvents, such as ethanol, to improve the solubility of the peptide(s) in the chosen propellant.

Active peptides of the invention may be delivered through inhaling devices suitable for dry powder inhalation, such as portable inhaler devices and the like. In such dry powder formulations, the active peptide(s) of the invention can be used either alone or in combination with a carrier, such as lactose, mannitol, or glucose. The selection of carrier is not critical, provided that the physiological action of the peptide(s) of the invention is substantially unimpaired. Other additives may also be included in powder formulations as required e.g. to maintain stability etc. Again, such additives should be such so as not to substantially interfere with the physiological and hence therapeutic effect of the peptide(s) of the invention. The inhaling device can be of any type known in the art, such as a single dose inhaler having a predetermined dose or a multi-dose inhaler wherein the dose is measured by a metering unit within the inhaler or is delivered from an assembly of predetermined doses.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient. Such formulations suitably comprise a solution of a pharmaceutically and pharmacologically acceptable acid addition salt of a peptide(s) of the invention that is isotonic with the blood of the recipient.

Useful formulations also comprise concentrated solutions or solids containing peptide(s) of the invention which upon dilution with an appropriate solvent give a solution for parenteral administration as above.

The invention will now be illustrated by the following non-limiting examples.
It is to be understood that where no group is shown at the N- and C- terminals of peptides of the invention herein described that the N-terminal is in the amino form (NH$_2$) and that the C-terminal is in the carboxyl form (-COOH).

In the synthetic examples below:

Peptides of the present invention were prepared using standard solid phase sequential coupling techniques on a Millipore 9050 automatic peptide synthesizer (for further information about this technique see for example Jones, J. The Chemical Synthesis of Peptides, pp 132-156, first edition, Oxford University Press, 1991 and R. Epton (ed) Innovation and Perspectives in Solid Phase Synthesis, SPCC (UK) Ltd 1990).

Oxidation of thiols into disulfides was accomplished using general oxidation techniques (e.g. Andreu, D et al. Methods in Molecular Biology, Vol 35: Peptide Synthesis Protocols edited by M.W. Pennington and B.M. Dunn Humana Press Inc., Totowa, NJ.).

The C-terminal aminoacid was purchased coupled to a resin which consisted of a crosslinked polystyrene backbone grafted with polyethylene glycol chains, functionalized with either the linker p-hydroxymethyl phenoxyacetic acid (TentaGel S PHB-Aaa-Fmoc, Sheppard, R.C., Williams, B.J. Acid-labile Resin Linkage Agents for Use in Solid Phase Peptide Synthesis. Int. J. Peptide Protein Res. 1982, 20, 451-454) from RAPP Polymere. N$^\text{a}$-Fmoc-protected L-amino acid pentafluorophenyl esters used were purchased from Bachem and Biosearch. DMF and 20% piperidine/DMF in peptide reagent quality was purchased from Biosearch. The coupling reagent 1-hydroxybenzotriazole (HOBT) came from Fluka. Synthesis were performed on a Millipore 9050 Plus PepSynthesizer.

In the Examples below ox denotes oxidised. The amino acid units are in L-form unless otherwise specified.
**Example 1:** Synthesis of Leu Ala Phe Glu Pro Cys Met (SEQ. ID. NO. 3)

The C-terminal aminoacid Tentagel S PHB-Met-Fmoc (0.90 g, 0.23 mmol/g, 0.2 mmol) on resin, drypacked in the synthesizer column, was allowed to swell in DMF for 30 minutes. The synthesizer worked with consecutive deblocking, washing and coupling cycles consisting of 8 min recycling with 20% piperidine/DMF for each Fmoc-deblocking followed, after wash, by activation of the N\(^{\text{a}}\)-Fmoc-protected L-amino acid pentafluorophenyl ester (0.8 mmol) with HOBT (0.9 mmol). Where pentafluorophenyl esters could not be obtained the N\(^{\text{a}}\)-Fmoc-Aaa-OH derivative was used which was coupled to aminoacids on the resin with HOBT (0.9 mmol) and DIPCDI (0.9 mmol). The activated amino acids were added to the column and recycled 30 min each. The synthesizer ended the synthesis with a deblocking of the N-terminal Fmoc-group and a final wash with DMF. The resulting peptide on the resin was transferred to a sintered glass funnel where it was washed twice with MeOH (2 x 10 ml) and three times with CH\(_2\)Cl\(_2\) (3 x 10 ml). The resin was allowed to dry under vacuum over night after which the peptide was side chained deprotected and cleaved from the resin using ethanedithiol/TFA 5/95 (20 ml) at room temperature for 3 h. The resin was filtered off and washed with 3 x 10 ml of acetic acid. The combined acidic fraction was evaporated after which the residue was triturated 3 times with ether.

The crude peptide was dissolved in H\(_2\)O/CH\(_3\)CN 1/1 and lyophilized. The resulting material was purified on HPLC using a Gilson 305 and 306 HPLC system with a Kromasil 100-5C18 25 cm x 20 mm id reversed phase column (0.1% TFA/CH\(_3\)CN - 0.1% TFA/H\(_2\)O 90 - 10, 10 ml/min, 220 nm). The combined HPLC fraction was lyophilized leaving 68 mg of the title compound.

Synthesis of Examples 2 - 14 below was accomplished following a similar protocol as per Example 1. The products obtained were as follows:

**Example 2:** Leu Leu Pro Gly Pro Cys Met (SEQ. ID. NO. 12)
Example 3: Met Ala Pro Ala Pro Cys Trp (SEQ. ID. NO. 13)
Example 4: Ala Leu Tyr Ala Pro Cys Met (SEQ. ID. NO. 14)
Example 5: Val Leu Trp Glu Pro Cys Trp (SEQ. ID. NO. 4)
Example 6: Met Leu Phe Ser Pro Cys Trp (SEQ. ID. NO. 5)
Example 7: Leu Leu Cys Gly Pro Ala Ile (SEQ. ID. NO. 15)
Example 8: Leu Cys Phe Gly Pro Ala Ile (SEQ. ID. NO. 16)
Example 9: Leu Arg Pro Arg Cys Arg Pro Ile (SEQ. ID. NO. 17)
Example 10: Ala Gly Tyr Cys Pro Thr Met Thr (SEQ. ID. NO. 18)
Example 11: Pro Gln Val Val Cys Asn Tyr Arg (SEQ. ID. NO. 19)
Example 12: Gln Cys Ala Leu Cys Arg (SEQ. ID. NO. 20)
Example 13: Ala Asn Phe Cys Ala Gly Ala Cys Pro Tyr Leu Trp (SEQ. ID. NO. 21)
Example 14: Ile Val Lys Ser Cys Lys (SEQ. ID. NO. 22)

Example 15: (Leu Leu Cys Gly Pro Ala Ile)$_2$ (SEQ. ID. NO. 15) (ox, dimer)

Leu Leu Cys Gly Pro Ala Ile (SEQ. ID. NO. 15) (69mg, 0.1 mmol) was prepared following the protocol as per Example 1. The peptide was dissolved in 5% aqueous acetic acid (25 ml) and the pH of the solution was adjusted to 6 with ammonium carbonate. Dimethylsulfoxide (5 ml) was added and the mixture was stirred at room temperature for 16 h. Upon completion, the reaction mixture was concentrated in vacuo until ca 5 ml remained. The crude product was purified on HPLC using a Gilson 305 and 306 HPLC system with a Kromasil 100-5C18 25 cm x 20 mm id reversed phase column (0.1% TFA/CH$_3$CN - 0.1% TFA/H$_2$O 90 - 10, 10 ml/min, 220 nm). The combined HPLC fraction was lyophilised to give the title peptide as a white powder.

Synthesis of Examples 16 - 24 below was accomplished following a similar protocol as per Example 15. The products obtained were as follows:

Example 16: (Leu Cys Phe Gly Pro Ala Ile)$_2$ (SEQ. ID. NO. 16) (ox, dimer)
Example 17: (Met Leu Phe Ser Pro Cys Trp)$_2$ (SEQ. ID. NO. 5) (ox, dimer)
Example 18: (Ala Val Pro Glu Pro Cys Phe)$_2$ (SEQ. ID. NO. 6) (ox, dimer)
Example 19: (Val Leu Trp Glu Pro Cys Trp)$_2$ (SEQ. ID. NO. 4) (ox, dimer)
Example 20: (Ala Leu Tyr Ala Pro Cys Met)$_2$ (SEQ. ID. NO. 14) (ox, dimer)
Example 21: (Met Met Tyr Glu Pro Cys Tyr)$_2$ (SEQ. ID. NO. 7) (ox, dimer)
Example 22: (Ala Ala Trp Ser Pro Cys Met)$_2$ (SEQ. ID. NO. 23) (ox, dimer)
Example 23: (Leu Leu Pro Gly Pro Cys Met)$_2$ (SEQ. ID. NO. 12) (ox, dimer)
Example 24: (Val Ala Tyr Gly Pro Cys Trp)$_2$ (SEQ. ID. NO. 8) (ox, dimer)

Synthesis of Examples 25 - 28 below was accomplished following a similar protocol as per Example 1. The products obtained were as follows:

Example 25: Leu Arg Pro Arg Cys Arg Pro (SEQ. ID. NO. 9)
Example 26: Pro Gln Val Val Cys Asn Tyr (SEQ. ID. NO. 10)
Example 27: Arg Gly Tyr Cys Pro Tyr (SEQ. ID. NO. 11)
Example 28: Gln Cys Ala Leu (SEQ. ID. NO. 24)

Example 29: Delayed Type Hypersensitivity (DTH) Test

This test was used to show immunomodulatory activity.

The ability of the peptides according to the invention to modulate immune responses can be illustrated by their effect in the delayed type hypersensitivity (DTH) test in mice. The DTH test is used to illustrate immunomodulation, the protocol for which is described, for example, by Carlsten H., et al (1986) Int. Arch. Allergy Appl. Immunol 81:322, herein incorporated by reference. The peptides were tested at one or more of the following dosages: 0.0003 µmol/kg, 0.003 µmol/kg, 0.03 µmol/kg, 0.3 µmol/kg, and 3.0 µmol/kg.

Male and female Balb/c mice were obtained from Bomholtsgaard (Denmark) with a weight of 18-20 grams each. 4-Ethoxymethylene-2-phenyloxazolin-5-one (OXA) (Sigma Chemicals) was used as the antigen in the DTH test.
The mice were sensitized, Day 0, by epicutaneous application of 150 μl of an absolute ethanol-acetone (3:1) solution containing 3% OXA on the shaved abdomen. Treatment with peptides according to the invention, or vehicle (phosphate buffer, pH 7.4, containing a mixture of Buffer A and Buffer B in the proportion 63%:37% B (Buffer A: Na₂HPO₄, 0.89 g/100ml, EDTA 0.05 g/100ml; Buffer B: NaH₂PO₄, 0.69 g/100ml, EDTA 0.05 g/100ml) was initiated by oral feeding immediately after sensitization and continued once daily (a.m) until Day 6. Seven days after sensitization, both ears of all mice were challenged on both sides by topical application of 20 μl 1% OXA dissolved in peanut oil. Ear thickness was measured prior to and at 24hrs or 48 hrs after challenge using an Oditester spring calliper. Challenges and measurements were performed under light pentobarbital anaesthesia.

The intensity of the DTH reactions was measured according to the method described by van Loveren H., et al (1984) J. Immunol. Methods 67: 311 and expressed according to the formula: T_{48}/T_{0} μm units, where t0, t24 and t48 represent the ear thickness at time 0, +24hrs or +48 hrs after challenge respectively, in individual tests (T). The results are expressed as the mean +/- S.E.M.. The level of significance between means of the groups is obtained by Student's two-tailed t-test. The immunomodulating effect of the peptide is reflected in a significant difference in the increase or decrease in ear thickness as compared to the control (phosphate buffer).

The peptides of Examples 1 to 19 and 21 to 28 were tested using the DTH test. Some of the products were found to exhibit very good effect, good effect or to be effective in this particular test and some were found to exhibit little toward no effect compared with the control of this particular test.
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
   (A) NAME: ASTRA AKTIEBOLAG
   (B) STREET: Vastra Malarehamnen 9
   (C) CITY: Sodertalje
   (E) COUNTRY: Sweden
   (F) POSTAL CODE (ZIP): S-151 85

(ii) TITLE OF INVENTION: CYSTEINE-CONTAINING PEPTIDES

(iii) NUMBER OF SEQUENCES: 24

(iv) COMPUTER READABLE FORM:
   (A) MEDIUM TYPE: Floppy disk
   (B) COMPUTER: IBM PC compatible
   (C) OPERATING SYSTEM: PC-DOS/MS-DOS
   (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
   APPLICATION NUMBER: WO na

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 4 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ala Pro Cys Trp

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gly Pro Cys Met

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Leu Ala Phe Glu Pro Cys Met

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Val Leu Trp Glu Pro Cys Trp

1      5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Leu Phe Ser Pro Cys Trp

1      5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

 Ala Val Pro Glu Pro Cys Phe

1      5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
    Met Met Tyr Glu Pro Cys Tyr
    1  5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
    Val Ala Tyr Gly Pro Cys Trp
    1  5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
Leu Arg Pro Arg Cys Arg Pro
1  5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 7 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Pro Gln Val Val Cys Asn Tyr
1  5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 6 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Arg Gly Tyr Cys Pro Tyr
1  5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 7 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Leu Leu Pro Gly Pro Cys Met

1  5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Ala Pro Ala Pro Cys Trp

1  5

30  (2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ala Leu Tyr Ala Pro Cys Met

1  5

45
(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
Leu Leu Cys Gly Pro Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
Leu Cys Phe Gly Pro Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
Leu Arg Pro Arg Cys Arg Pro Ile
1  5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 8 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
Ala Gly Tyr Cys Pro Thr Met Thr
1  5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 8 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
Pro Gln Val Val Cys Asn Tyr Arg
1  5

(2) INFORMATION FOR SEQ ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 6 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 12 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 6 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Ile Val Lys Ser Cys Lys

1 5

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

 Ala Ala Trp Ser Pro Cys Met
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Gln Cys Ala Leu
1
CLAIMS

1. A physiologically active peptide of formula (I):

\[ \text{A(R}_1 \text{)}_{\text{x}} \text{R}_2 \text{CysR}_3 \text{B} \quad (\text{I}) \]

wherein

each \( \text{A} \) is independently selected from \( H \), a protecting group, or at least one amino acid residue independently selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

\( \text{x} \) is 0 (\( R_1 \) being absent), and

\( \text{R}_2 \) and \( \text{R}_3 \) are residues of amino acids chosen from one of the following combinations (a) and (b) of amino acids

15

(a) \( \text{R}_2 \) leucine (Leu), \( \text{R}_3 \) phenylalanine (Phe)

(b) \( \text{R}_2 \) glutamine (Gln), \( \text{R}_3 \) alanine (Ala), or

\( \text{x} \) is 1, and

20

\( \text{R}_1 \) and \( \text{R}_2 \) and \( \text{R}_3 \) are residues of amino acids chosen from one of the following combinations (c) and (f) to (s) of amino acids

(c) \( \text{R}_1 \) glutamic acid (Glu), \( \text{R}_2 \) proline (Pro), \( \text{R}_3 \) methionine (Met)

(f) \( \text{R}_1 \) alanine (Ala), \( \text{R}_2 \) proline (Pro), \( \text{R}_3 \) methionine (Met)

(g) \( \text{R}_1 \) glutamic acid (Glu), \( \text{R}_2 \) proline (Pro), \( \text{R}_3 \) tryptophan (Trp)

(h) \( \text{R}_1 \) serine (Ser), \( \text{R}_2 \) proline (Pro), \( \text{R}_3 \) tryptophan (Trp)

(i) \( \text{R}_1 \) leucine (Leu), \( \text{R}_2 \) leucine (Leu), \( \text{R}_3 \) glycine (Gly)

(j) \( \text{R}_1 \) proline (Pro), \( \text{R}_2 \) arginine (Arg), \( \text{R}_3 \) arginine (Arg)
(k) \( R_1 \) glycine (Gly), \( R_2 \) tyrosine (Tyr), \( R_3 \) proline (Pro)
(l) \( R_1 \) valine (Val), \( R_2 \) valine (Val), \( R_3 \) asparagine (Asn)
(m) \( R_1 \) alanine (Ala), \( R_2 \) leucine (Leu), \( R_3 \) arginine (Arg)
(n) \( R_1 \) glycine (Gly), \( R_2 \) alanine (Ala), \( R_3 \) proline (Pro)
(o) \( R_1 \) lysine (Lys), \( R_2 \) serine (Ser), \( R_3 \) lysine (Lys)
(p) \( R_1 \) glutamic acid (Glu), \( R_2 \) proline (Pro), \( R_3 \) phenylalanine (Phe)
(q) \( R_1 \) glutamic acid (Glu), \( R_2 \) proline (Pro), \( R_3 \) tyrosine (Tyr)
(r) \( R_1 \) serine (Ser), \( R_2 \) proline (Pro), \( R_3 \) methionine (Met)
(s) \( R_1 \) glycine (Gly), \( R_2 \) proline (Pro), \( R_3 \) tryptophan (Trp),

with the provisos that, when \( x \) is 0 (\( R_1 \) is absent), \( A \) is \( H \) or a protecting group, and, when \( R_1 \) is glycine (Gly), \( A \) is said at least one amino acid residue,

each \( B \) is independently selected from the group consisting of \( \text{OH, NH}_2 \), an oxygen or a nitrogen carrying a protecting group, or at least one amino acid residue selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

the entire peptide sequence containing up to 20 amino acid residues; or

a homo- or heterodimer thereof.

2. A peptide according to claim 1 wherein

\( x \) is 0 (\( R_1 \) being absent), and

\( R_2 \) and \( R_3 \) are residues of amino acids of the following combination (b) of amino acids

(b) \( R_2 \) glutamine (Gln), \( R_3 \) alanine (Ala), or
x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c), (f) to (l) and (p) to (s) of amino acids

(c)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(f)  R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h)  R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i)  R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j)  R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k)  R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l)  R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(p)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r)  R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s)  R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),

with the provisos that, when x is 0 (R₁ is absent), A is H or a protecting group, and, when R₁ is glycine (Gly), A is said at least one amino acid residue.

3. A peptide according to claim 1, wherein:

x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c), (g), (h), (j) to (l), (p), (q) and (s) of amino acids

(c)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(g)  R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h)  R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(j) $R_1$ proline (Pro), $R_2$ arginine (Arg), $R_3$ arginine (Arg)
(k) $R_1$ glycine (Gly), $R_2$ tyrosine (Tyr), $R_3$ proline (Pro)
(l) $R_1$ valine (Val), $R_2$ valine (Val), $R_3$ asparagine (Asn)
(p) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ phenylalanine (Phe)

(q) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ tyrosine (Tyr)
(s) $R_1$ glycine (Gly), $R_2$ proline (Pro), $R_3$ tyrosine (Tyr),

with the proviso that, when $R_1$ is glycine (Gly), A is said at least one amino acid residue.

4. A peptide according to claim 1 wherein

x is 1, and

$R_1$, $R_2$ and $R_3$ are residues of amino acids chosen from one of the following combinations
(c) and (j) to (l) of amino acids

(c) $R_1$ glutamic acid (Glu), $R_2$ proline (Pro), $R_3$ methionine (Met)
(j) $R_1$ proline (Pro), $R_2$ arginine (Arg), $R_3$ arginine (Arg)
(k) $R_1$ glycine (Gly), $R_2$ tyrosine (Tyr), $R_3$ proline (Pro)
(l) $R_1$ valine (Val), $R_2$ valine (Val), $R_3$ asparagine (Asn),

with the proviso that, when $R_1$ is glycine (Gly), A is said at least one amino acid residue.

5. A peptide according to any of claims 1 to 4 in the form of a cyclised monomer in which one cysteine residue forms an intramolecular bridge with another cysteine residue.

6. A peptide according to any of claims 1 to 5 wherein the or each peptide sequence is from 4 to 20 amino acid residues in length.

7. A peptide according to any of claims 1 to 5 wherein the or each peptide sequence is from 4 to 15 amino acid residues in length.

8. A peptide according to claim 1 of formula:
Leu Ala Phe Glu Pro Cys Met  (SEQ. ID. NO. 3)
Val Leu Trp Glu Pro Cys Trp  (SEQ. ID. NO. 4)
Met Leu Phe Ser Pro Cys Trp  (SEQ. ID. NO. 5)
(Met Leu Phe Ser Pro Cys Trp)₂  (SEQ. ID. NO. 5)
(Ala Val Pro Glu Pro Cys Phe)₂  (SEQ. ID. NO. 6)
(Met Met Tyr Glu Pro Cys Tyr)₂  (SEQ. ID. NO. 7)
(Val Ala Tyr Gly Pro Cys Trp)₂  (SEQ. ID. NO. 8)
Leu Arg Pro Arg Cys Arg Pro  (SEQ. ID. NO. 9)
Pro Gin Val Val Cys Asn Tyr  (SEQ. ID. NO. 10)
Arg Gly Tyr Cys Pro Tyr  (SEQ. ID. NO. 11)

9. A peptide according to claim 1 specifically identified herein.
10. A peptide according to any of claims 1 to 9 free from an added transport agent.
11. Acid addition salts of peptides according to any of claims 1 to 10.
12. Acid addition salts according to claim 11 selected from the group consisting of salts of hydrochloric, hydrobromic, nitric, perchloric, sulphuric, citric, tartaric, phosphoric, lactic, benzoic, glutamic, oxalic, aspartic, pyruvic, acetic, succinic, fumaric, maleic, oxaloacetic, isethionic, stearic, phthalic, methanesulphonic, p-toluene sulphonic, benzenesulphonic, lactobionic, glucuronic, and trifluoroacetic acids.
13. Base salts of peptides according to any of claims 1 to 10.
14. Base salts according to claim 13 selected from the group consisting of alkali metal and alkaline earth salts, organic base salts, and amino acid salts.
15. An oral dosage form comprising an immunomodulatory peptide according to any of claims 1 to 14.
16. An oral dosage form according to claim 15 wherein the amount of the immuno-modulatory peptide needed to induce an observable level of modulated immune response in a mammal when administered orally is less than the amount of the same immunomodulatory peptide when administered parenterally which is needed to achieve a similar observable level of modulated immune response in the said mammal.
17. An oral dosage form according to claim 15 or 16 not including an added transport agent.

18. An intra-tracheal dosage form comprising an immunomodulatory peptide according to any of claims 1 to 14.

19. An intra-tracheal dosage form according to claim 18 wherein the amount of the immunomodulatory peptide needed to induce an observable level of modulated immune response in a mammal when administered intra-tracheally is less than the amount of the same immunomodulatory peptide when administered parenterally which is needed to achieve a similar observable level of modulated immune response in the said mammal.

20. An intra-tracheal dosage form according to claim 18 or 19 not including an added transport agent.

21. A nasal dosage form comprising an immunomodulatory peptide according to any of claims 1 to 14.

22. A nasal dosage form according to claim 21 wherein the amount of the immuno-modulatory peptide needed to induce an observable level of modulated immune response in a mammal when administered nasally is less than the amount of the same immunomodulatory peptide when administered parenterally which is needed to achieve a similar observable level of modulated immune response in the said mammal.

23. A nasal dosage form according to claim 22 not including an added transport agent.

24. Pharmaceutical formulation comprising at least one peptide according to any of claims 1 to 14 or a salt thereof together with a pharmaceutically acceptable carrier therefor.

25. Pharmaceutical formulation according to claim 24 wherein the pharmaceutical formulation is for oral administration.

26. A peptide according to any of claims 1 to 14 or a salt thereof for use in therapy.

27. A peptide according to any of claims 1 to 14 or a salt thereof for use in cancer therapy.
28. A peptide according to any of claims 1 to 14 or a salt thereof for use in rheumatoid arthritis therapy.

29. A peptide according to any of claims 1 to 14 or a salt thereof in an oral dosage form for use in therapy.

30. A peptide according to any of claims 1 to 14 or a salt thereof in an oral dosage form for use in cancer therapy.

31. A peptide according to any of claims 1 to 14 or a salt thereof in an oral dosage form for use in autoimmune disease therapy.

32. Use of a physiologically active peptide according to any of claims 1 to 14 or a salt thereof in the preparation of a medicament suitable for the treatment of disease.

33. Use of a physiologically active peptide of formula (I):

\[
A^\left(\mathbf{R_1}\right)^x - R_2 - \text{Cys} - R_3 - B
\]

(1)

wherein

each A is independently selected from H, a protecting group, or at least one amino acid residue independently selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

\[x\] is 0 (\(R_1\) being absent), and

\(R_2\) and \(R_3\) are residues of amino acids chosen from one of the following combinations (a) and (b) of amino acids

(a) \(R_2\) leucine (Leu), \(R_3\) phenylalanine (Phe)

(b) \(R_2\) glutamine (Gln), \(R_3\) alanine (Ala), or

\[x\] is 1, and
R₁ and R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(d) R₁ glycine (Gly), R₂ proline (Pro), R₃ methionine (Met)
(e) R₁ alanine (Ala), R₂ proline (Pro), R₃ tryptophan (Trp)
(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(m) R₁ alanine (Ala), R₂ leucine (Leu), R₃ arginine (Arg)
(n) R₁ glycine (Gly), R₂ alanine (Ala), R₃ proline (Pro)
(o) R₁ lysine (Lys), R₂ serine (Ser), R₃ lysine (Lys)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r) R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),

with the provisos that, when x is 0 (R₁ is absent), A is H or a protecting group, and, when R₁ is glycine (Gly), A is said at least one amino acid residue,

each B is independently selected from the group consisting of OH, NH₂, an oxygen or a nitrogen carrying a protecting group, or at least one amino acid residue selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;
the entire peptide sequence containing up to 20 amino acid residues; or

a homo- or heterodimer thereof, or a salt thereof in the preparation of a medicament suitable for the treatment of a disease requiring immunomodulatory treatment.

34. Use of a physiologically active peptide as defined in claim 33 or a salt thereof in the preparation of a medicament suitable for the treatment of cancer.

35. Use of a physiologically active peptide as defined in claim 33 or a salt thereof in the preparation of a medicament suitable for the treatment of autoimmune disease.

36. Use of a physiologically active peptide as defined in claim 33 or a salt thereof free from an added transport agent in the preparation of a medicament suitable for the treatment of rheumatoid arthritis.

37. Use according to any of claims 33 to 36 wherein, in the peptide,

x is 0 (R₁ being absent), and

R₂ and R₃ are residues of amino acids of the following combination (b) of amino acids

(b) R₂ glutamine (Gln), R₃ alanine (Ala), or

x is 1, and

R₁, R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c) to (l) and (p) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)

(d) R₁ glycine (Gly), R₂ proline (Pro), R₃ methionine (Met)

(e) R₁ alanine (Ala), R proline (Pro), R₃ tryptophan (Trp)

(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)

(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r) R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),

with the provisos that, when x is 0 (R₁ is absent), A is H or a protecting group, and, when
R₁ is glycine (Gly), A is said at least one amino acid residue.

38. Use according to any of claims 33 to 36 wherein the peptide or salt is as
defined in any of claims 1 to 14.

39. Pharmaceutical formulation comprising at least one peptide or salt as
defined in any one of claims 33 to 38 together with a pharmaceutically acceptable carrier
therefor but not including an added transport agent.

40. A method of inducing an immunomodulatory response in a mammal which
comprises identifying a mammal in need of immunomodulatory response; and
administering to the epithelial cell lining of the mammal a dose of a physiologically active
peptide of formula (I):

\[ A₋(R₁)₋R₂₋\text{Cys}₋R₃₋B \quad (I) \]

wherein

each A is independently selected from H, a protecting group, or at least one amino acid
residue independently selected from the group of amino acid residues having aliphatic side
chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary
amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

x is 0 (R₁ being absent), and
R₂ and R₃ are residues of amino acids chosen from one of the following combinations (a) and (b) of amino acids

(a) R₂ leucine (Leu), R₃ phenylalanine (Phe)
(b) R₂ glutamine (Gln), R₃ alanine (Ala), or

x is 1, and

R₁ and R₂ and R₃ are residues of amino acids chosen from one of the following combinations (c) to (s) of amino acids

(c) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ methionine (Met)
(d) R₁ glycine (Gly), R₂ proline (Pro), R₃ methionine (Met)
(e) R₁ alanine (Ala), R₂ proline (Pro), R₃ tryptophan (Trp)
(f) R₁ alanine (Ala), R₂ proline (Pro), R₃ methionine (Met)
(g) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tryptophan (Trp)
(h) R₁ serine (Ser), R₂ proline (Pro), R₃ tryptophan (Trp)
(i) R₁ leucine (Leu), R₂ leucine (Leu), R₃ glycine (Gly)
(j) R₁ proline (Pro), R₂ arginine (Arg), R₃ arginine (Arg)
(k) R₁ glycine (Gly), R₂ tyrosine (Tyr), R₃ proline (Pro)
(l) R₁ valine (Val), R₂ valine (Val), R₃ asparagine (Asn)
(m) R₁ alanine (Ala), R₂ leucine (Leu), R₃ arginine (Arg)
(n) R₁ glycine (Gly), R₂ alanine (Ala), R₃ proline (Pro)
(o) R₁ lysine (Lys), R₂ serine (Ser), R₃ lysine (Lys)
(p) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ phenylalanine (Phe)
(q) R₁ glutamic acid (Glu), R₂ proline (Pro), R₃ tyrosine (Tyr)
(r) R₁ serine (Ser), R₂ proline (Pro), R₃ methionine (Met)
(s) R₁ glycine (Gly), R₂ proline (Pro), R₃ tryptophan (Trp),
with the provisos that, when \( x \) is 0 (\( R_1 \) is absent), \( A \) is \( H \) or a protecting group, and, when \( R_1 \) is glycine (Gly), \( A \) is said at least one amino acid residue,

each \( B \) is independently selected from the group consisting of \( \text{OH}, \text{NH}_2 \), an oxygen or a nitrogen carrying a protecting group, or at least one amino acid residue selected from the group of amino acid residues having aliphatic side chains, aliphatic hydroxyl side chains, basic side chains, acidic side chains, secondary amino groups, amide side chains, aromatic side chains, and sulphur containing side chains;

the entire peptide sequence containing up to 20 amino acid residues; or

a homo- or heterodimer thereof, or a salt thereof enough to induce said modulated immune response and thereby a therapeutic effect.

41. A method according to claim 40 which comprises administering the physiologically active peptide or salt thereof to the MALT of the mammal a dose in an amount sufficient to induce a modulated immune response and thereby a therapeutic effect.

42. A method according to claim 40 or 41 wherein the physiologically active peptide is as defined in any of claims 1 to 14 and 37.

43. A method for the preparation of a pharmaceutical formulation comprising bringing into association at least one peptide according to any of claims 1 to 14 or at least one salt thereof, and a pharmaceutically acceptable carrier therefor.

44. A method of making a peptide according to any of claims 1 to 14 by a chemical process in which individual amino acid residues or fragments of peptides of the invention are joined to form peptide bonds and wherein protecting groups are employed at the beginning and/or end of the process.