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(54) Title: SYNTHETIC PEPTIDES AND METHODS OF USE FOR AUTOIMMUNE DISEASE THERAPIES
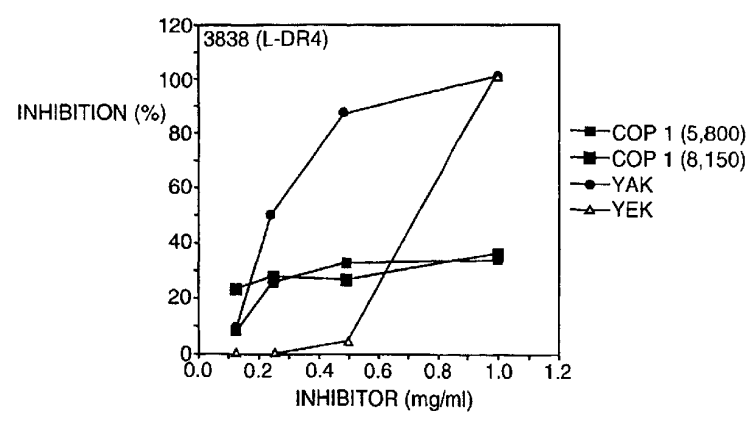
(57) Abstract

The invention provides heteropolymer compositions and peptide compositions, and methods of making and using therapeutic compositions comprising amino acid heteropolymers for treatment of a subject for an autoimmune or an inflammatory disease, the heteropolymer compositions made by solid state synthesis. The invention also provides kits for assaying binding of a composition to a water-soluble MHC protein.

Abstract

The invention provides peptide compositions, and
methods of making and using therapeutic compositions for
5   treatment of a subject for an autoimmune or an
inflammatory disease. The invention also provides kits
for assaying binding of a composition to a water-soluble
MHC protein.

SYNTHETIC PEPTIDES AND METHODS OF USE
FOR AUTOIMMUNE DISEASE THERAPIES

5   Technical Field

This invention provides in various embodiments a peptide composition having
defined sequences, and methods of making and using these compositions, for treatment of
autoimmune diseases. Purified MHC class II proteins are used to determine the motifs for
binding, and for binding in competition with test antigens, to identify peptide compositions
10   with potential therapeutic activity.

Background

An autoimmune disease results from an inappropriate immune response directed
against a self antigen (an autoantigen), which is a deviation from the normal state of self-
tolerance. Self-tolerance arises when the production of T cells and B cells capable of
15   reacting against autoantigens has been prevented by events that occur in the development of
the immune system during early life. The cell surface proteins that play a central role in
regulation of immune responses through their ability to bind and present processed
peptides to T cells are the major histocompatibility complex (MHC) molecules (Rothbard, J.
B., et al., 1991, *Annu. Rev. Immunol.* 9:527).

20   A number of therapeutic agents have been developed to treat autoimmune diseases,
including general anti-inflammatory drugs such as "super aspirins", for example, agents that
can prevent formation of low molecular weight inflammatory compounds by inhibiting a
cyclooxygenase; agents that can function by inhibiting a protein mediator of inflammation,
for example, by sequestering the inflammatory protein tumor necrosis factor (TNF) with an
25   anti-TNF specific monoclonal antibody or antibody fragment, or with a soluble form of the
TNF receptor; agents that target a protein on the surface of a T cell and generally prevent
interaction with an antigen presenting cell (APC) by inhibiting the CD4 receptor or the cell
adhesion receptor ICAM-1. However, compositions having natural folded proteins as
therapeutic agents can incur problems in production, formulation, storage, and delivery.
30   Several of these problems necessitate delivery to the patient in a hospital setting.

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An additional target for inhibition of an autoimmune response is the set of lymphocyte surface proteins MHC molecules, particularly a protein encoded by an MHC class II gene, for example, HLA-DR, -DQ and -DP. Each of the
5 MHC genes is found in a large number of alternative or allelic forms within a mammalian population. The genomes of subjects affected with certain autoimmune diseases, for example multiple sclerosis (MS) and rheumatoid arthritis (RA), are more likely to carry one or more characteristic
10 MHC class II alleles, to which that disease is linked.

RA is a common human autoimmune disease with a prevalence of about 1% among Caucasians (Harris, E.J. et al., 1997, *In Textbook of Rheumatology* 898-932), currently affecting 2.5 million Americans. RA is characterised by
15 chronic inflammation of the synovial joints and infiltration by activated T cells, macrophages and plasma cells, leading to a progressive destruction of the articular cartilage. It is the most severe form of joint disease. Inherited susceptibility to RA is strongly
20 associated with the affected subject having at the MHC class II DRB1 locus the allele DRB1*0401, DRB1*0404, or DRB1*0405 or the DRB1*0101 allele. The nature of the autoantigen(s) in RA is poorly understood, although collagen type II (CII) is a prominent candidate. An
25 immunodominant T cell epitope in collagen type II corresponding to residues 261-273 has been identified (Fugger, L., et al., 1996, *Eur. J. Immunol.* 26:928-933).

It would be desirable to identify agents that were able to bind specifically to one or more of the linked MHC
30 class II molecules and thereby to inhibit an inappropriate immune response. An agent that interacts and binds relatively nonspecifically to several MHC class II molecules is Copolymer I (Cop 1), a synthetic amino acid heteropolymer that was shown to be capable of suppressing
35 experimental allergic encephalomyelitis (EAE; Sela, M., R. Arnon, et al., 1990, *Bull. Inst. Pasteur* (Paris)), which can be induced in the mouse and is a model for MS. Cop 1

which is poly (Y,E,A,K), indicated herein "YEAK" using the
one letter amino acid code (see infra; Y represents
tyrosine, E glutamic acid, A alanine, and K lysine) has
been used to treat relapsing forms of MS but does not
5 suppress the disease entirely (Bornstein, M. B., et al.,
1987, *N. Engl, J. Med.* 317:408; Johnson, KP., et al.,
1995, *Neurology* 45:1268).

There is a need for improved treatments for
autoimmune diseases. A potential source of such
10 treatments would be to identify agents that bind
selectively to a purified MHC class II allele protein
molecule *in vitro*, particularly to a protein which is a
product of an MHC class II allele that is associated with
an autoimmune disease. In addition, the agent should also
15 bind to that protein as it occurs on the surfaces of
antigen presenting cells *in vivo*, and thereby can block,
anergize, or inactivate T cells that are responsible for
the autoimmune disease.

All references, including any patents or patent
20 applications, cited in this specification are hereby
incorporated by reference. No admission is made that any
reference constitutes prior art. The discussion of the
references states what their authors assert, and the
applicants reserve the right to challenge the accuracy and
25 pertinency of the cited documents. It will be clearly
understood that, although a number of prior art
publications are referred to herein, this reference does
not constitute an admission that any of these documents
forms part of the common general knowledge in the art, in
30 Australia or in any other country.

For the purposes of this specification it will be
clearly understood that the word "comprising" means
"including but not limited to", and that the word
"comprises" has a corresponding meaning.
35

Summary

The present invention provides a synthetic peptide

having an amino acid sequence comprising at least three
residues selected from the group of amino acids consisting
of aromatic acids, negatively charged amino acids,
positively charged amino acids, and aliphatic amino acids,
5   the synthetic peptide being at least seven amino acid
residues in length and capable of binding to an MHC class
II protein associated with an autoimmune disease.

     The present invention further provides a composition
comprising a synthetic peptide having therapeutic activity
10   in a subject suffering from an autoimmune disease, and a
pharmaceutically acceptable carrier, wherein the peptide
has an amino acid sequence having at least one of each of
amino acids glutamic acid, lysine, and alanine and an
amino acid selected from the group consisting of tyrosine,
15   valine, and phenylalanine.

     The present invention further provides an isolated
peptide having a sequence selected from the group
consisting of: AKEYAAAAAAKAAAA (SEQ ID NO: 7),
AAEYAAAAAAKAAAA (SEQ ID NO: 12), AAKYAEAAAAKAAAA (SEQ ID
20   NO: 15), and EAKYAAAAAAKAAAA (SEQ ID NO: 18).

     The present invention further provides an isolated
peptide having a sequence selected from the group
consisting of: AEKYAAAAAAKAAAA (SEQ ID NO: 6),
AKEYAAAAAAKAAAA (SEQ ID NO: 7), KEAYAAAAAAKAAAA (SEQ ID
25   NO: 10), AEEYAAAAAAKAAAA (SEQ ID No: 11), AAEYAAAAAAKAAAA
(SEQ ID NO: 12), EKAYAAAAAAKAAAA (SEQ ID NO: 13),
AAKYEAAAAAKAAAA (SEQ ID NO: 14), AAKYAEAAAAKAAAA (SEQ ID
NO. 15), EAAYAAAAAAKAAAA (SEQ ID NO: 16), EKKYAAAAAAKAAAA
(SEQ ID NO: 17), EAKYAAAAAAKAAAA (SEQ ID NO: 18),
30   AKKYEAAAAAAAAAA (SEQ ID NO: 21), AAEYKAAAAAAAAA (SEQ ID
NO: 26), AAKYEAAAAAAAAA (SEQ ID NO: 28), AAKYAEAAAAAAAA
(SEQ ID NO: 29), AEYAKAAAAAAAAA (SEQ ID NO: 32),
AEKAYAAAAAAAAAA (SEQ ID NO: 33), AYKAEAAAAAAAAA (SEQ ID
NO: 35), and AKYAEAAAAAAAAA (SEQ ID NO: 36), the peptide
35   having high affinity for an MHC class II protein.

The present invention further provides an isolated
peptide having an amino acid sequence capable of
inhibiting an immune response in a subject to an
autoantigen, wherein a position in the amino acid sequence
5   of the peptide that corresponds to an antigen binding
pocket in a peptide binding groove of an MHC class II DR
protein is identified as a particular amino acid.

The present invention further provides a method for
obtaining an MHC class II binding motif amino acid
10   sequence in a mixture of synthetic peptide heteropolymers
having therapeutic activity in a subject, comprising:

      (a)   binding the mixture of synthetic
heteropolymers to MHC Class II protein molecules to form
heteropolymer-MHC protein complexes;

15   (b)   removing by peptidase enzyme digestion the
amino terminal amino acid residues of the heteropolymers
protruding from the heteropolymer -MHC protein complex to
align amino termini of the heteropolymers to the edge of
the MHC protein complexes; and

20   c)   eluting the aligned heteropolymers from the
MHC protein by dissociating the complexes to release the
amino terminal aligned heteropolymers having the binding
motif.

The present invention further provides a method for
25   identifying an MHC class II amino acid binding motif in a
mixture of synthetic peptide heteropolymer molecules,
wherein the MHC class II is associated with an autoimmune
disease, the heteropolymer molecules being a random
sequence having a particular molar ratio of component
30   amino acids, comprising:

      (a) binding the mixture of synthetic heteropolymer
molecules to purified recombinant MHC class II protein
molecules to form heteropolymer-MHC protein complexes;

      (b) removing by aminopeptidase enzyme digestion
35   amino terminal amino acid residues of heteropolymer
molecules protruding from the heteropolymer-MHC protein
complexes to produce resulting heteropolymers having

aligned amino termini and a consensus binding motif within MHC class II protein complexes, the binding motif being located at amino termini of the resulting heteropolymer molecules;

5   (c) eluting the resulting aligned heteropolymer molecules from the complexes by dissociating the complexes; and

(d) determining the amino acid sequence from the amino terminal of the eluted aligned heteropolymer

10   molecules, to identify the binding motif.

In one embodiment of the invention, a composition is provided which is a synthetic peptide having an amino acid sequence comprising at least three residues selected from the group of amino acids consisting of aromatic acids,

15   negatively charged amino acids, positively charged amino acids, and aliphatic amino acids, the synthetic peptide being at least seven amino acid residues in length and capable of binding to an MHC class II protein associated with an autoimmune disease. Thus the aromatic amino acid

20   is selected from the group consisting of tyrosine (Y), valine (V), and phenylalanine (F), the positively charged amino acid is lysine (K), and the sequence is selected from the group consisting of lysine-tyrosine (KY), lysine-valine (KV), and lysine-phenylalanine (KF). Further, the

25   embodiment of the invention provides a composition wherein the negatively charged amino acid residue is glutamic acid (E), and the sequence is selected from the group consisting of glutamic acid-lysine-tyrosine (EKY), glutamic acid-lysine-valine (EKV), and glutamic acid-

30   lysine-phenylalanine (EKV). Even further, in the provided composition the amino acid which is aliphatic is alanine (A), and the sequence is selected from the group of amino acid sequences consisting of glutamic acid-lysine-tyrosine-alanine (EKYA), glutamic acid-lysine-valine-

35   alanine (EKVA), and glutamic acid-lysine-phenylalanine-alanine (EKFA). The composition can further comprise an amino-terminal alanine, and the sequence is selected from

the group of amino acid sequences consisting of alanine-glutamic acid-lysine-tyrosine-alanine (AEKYA), alanine-glutamic acid-lysine-valine-alanine (AEKVA), and alanine-glutamic acid-lysine-phenylalanine-alanine (AEKFA). The

5   synthetic peptides that are the embodiments of the invention are capable of binding to an MHC class II protein associated with an autoimmune disease, for example, an arthritic condition, for example, rheumatoid arthritis. In another embodiment, the synthetic

peptide composition which is an embodiment of the invention has aliphatic amino acid which is alanine, and the amino acid sequence is selected from the group of sequences consisting of: lysine-glutamic acid-tyrosine-alanine (KEYA), lysine-tyrosine-alanine-glutamic acid (KYAE), lysine-glutamic acid-valine-alanine (KEVA), lysine-valine-

5   alanine-glutamic acid (KVAE), lysine-glutamic acid-phenylalanine-alanine (KEFA), and lysine-phenylalanine-alanine-glutamic acid (KFAE). In a further embodiment wherein the aliphatic amino acid is alanine (A), the amino acid sequence is selected from the group of amino acid sequences consisting of lysine-tyrosine-alanine-alanine (KYAA) or lysine-lysine-tyrosine-alanine (KKYA), lysine-valine-alanine-alanine (KVAA) or lysine-lysine-

10   valine-alanine (KKVA), lysine-phenylalanine-alanine-alanine (KFAA), and lysine-lysine-phenylalanine-alanine (KKFA). In this embodiment, the peptide can further comprise two alanine residues, and the sequence can be selected from the group of sequences consisting of alanine-lysine-tyrosine-alanine-glutamic acid (AKYAE), glutamic acid-alanine-lysine-tyrosine-alanine (EAKYA), alanine-lysine-valine-alanine-glutamic

15   acid (AKVAE); and glutamic acid-alanine-lysine-valine-alanine (EAKVA), and alanine-lysine-phenylalanine-alanine-glutamic acid (AKFAE); and glutamic acid-alanine-lysine-phenylalanine-alanine (EAKFA). The peptide composition of this embodiment of the invention can be 7-100 amino acid residues in length.

Another embodiment of the invention provides a composition which is a synthetic

20   peptide having therapeutic activity in a subject suffering from an autoimmune disease, and the amino acid sequence having at least one of each of amino acids glutamic acid, lysine, and alanine and an amino acid selected from the group consisting of tyrosine, valine, and phenylalanine. The composition can be a peptide which is 7-100 amino acids in length, for example, 7-50 amino acids in length, 7-25 amino acids in length, and 7-15

25   amino acids in length. The composition can be formulated as a unitary dosage in a pharmaceutically acceptable carrier, for example, a synthetic peptide which is substantially pure. An embodiment of the invention is a synthetic peptide having greater affinity for the antigen binding groove of an MHC class II protein associated with the autoimmune disease than a type II collagen 261-273 peptide. In a further example of

30   these embodiments, a composition is provided comprising an amino acid analog at the residue locations and in an amount protease degradation of the peptide in the subject.

Another embodiment of the invention is an isolated peptide composition having a

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sequence selected from the group consisting of: AKEYAAAAAAKAAAA (SEQ ID NO: 7), AAEYAAAAAAKAAAA (SEQ ID NO: 12), AAKYAEAAAAKAAAA (SEQ ID NO: 15), and EAKYAAAAAAKAAAA (SEQ ID NO: 18). A further embodiment of the invention is an example of one of the preceding isolated peptides in which the tyrosine

5   (Y) has been substituted by a valine (F) or a phenylalanine (F). Further, an embodiment of the invention can be an isolated peptide composition having a sequence selected from the group consisting of: AEKYAAAAAAKAAAA (SEQ ID NO:6), AKEYAAAAAAKAAAA (SEQ ID NO: 7), K EAYAAAAAAKAAAA (SEQ ID NO: 10), AEEYAAAAAAKAAAA (SEQ ID NO: 11), AAEYAAAAAAKAAAA (SEQ ID

10 NO: 12), EKAYAAAAAAKAAAA (SEQ ID NO: 13), AAKYEAAAAAKAAAA (SEQ ID NO: 14), AAKYAEAAAAKAAAA (SEQ ID NO: 15), EAAYAAAAAAKAAAA (SEQ ID NO: 16), EKKYAAAAAAKAAAA (SEQ ID NO: 17), EAKYAAAAAAKAAAA (SEQ ID NO: 18), AKKYEAAAAAAAAAA (SEQ ID NO: 21), AAEYKAAAAAAAAAA (SEQ ID NO: 26), AAKYEAAAAAAAAAA (SEQ ID

15 NO: 28), AAKYAEAAAAAAAAA (SEQ ID NO: 29), AEYAKAAAAAAAAAA (SEQ ID NO: 32), AEKAYAAAAAAAAAA (SEQ ID NO: 33), AYKAEAAAAAAAAAA (SEQ ID NO: 35), and AKYAEAAAAAAAAAA (SEQ ID NO: 36), the peptide having high affinity for an MHC class II protein. Yet another embodiment of the invention is an isolated peptide according to any of the preceding sequences in which the tyrosine (Y) has

20 been substituted by a valine (F) or a phenylalanine (F).

    Another embodiment of the invention provides an isolated peptide composition having an amino acid sequence capable of inhibiting immune response in a subject to an autoantigen, wherein a position in the amino acid sequence of the peptide that corresponds to an antigen binding pocket in a peptide binding groove of an MHC class II

25 DR protein is identified as a particular amino acid. For example, an isolated peptide composition is provided wherein the autoantigen is associated with a condition selected from the group consisting of multiple sclerosis and arthritis. The MHC class II protein can be selected from the group consisting of an HLA-DR1 protein, an HLA-DR4 protein. In another embodiment, the MHC class II protein is MHC class II HLA-DR2.   An

30 embodiment of the invention provides an isolated peptide, wherein the amino acid residue in the position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is selected from the group consisting of a tyrosine, a valine, and a

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phenylalanine. This embodiment further provides an isolated peptide composition wherein the amino acid residue in a first amino acid position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is alanine. The embodiment further provides an isolated peptide composition, wherein the amino acid

5   residue located eight residues beyond the first amino acid position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is selected from the group consisting of lysine and alanine residues, and the amino acid residue that corresponds to the P1 pocket is selected from the group consisting of tyrosine, valine, and phenylalanine.

10   Another example of this invention provides a pharmaceutical preparation comprising a first peptide sequence and a second peptide sequence, wherein the composition is a mixture of first peptide sequence and the second peptide sequence, the first sequence having a lysine residue and the second sequence having an alanine residue at the amino acid position corresponding to eight residues beyond the amino acid

15   corresponding to the P1 pocket in the MHC class II peptide binding groove.

The autoimmune disease is selected from the group consisting of: multiple sclerosis, myasthenia gravis, Hashimoto's disease, systemic lupus erythematosis, uveitis, Guillain-Barre' syndrome, Grave's disease, idiopathic myxedema, autoimmune oophoritis, chronic immune thrombocytopenic purpura, colitis, diabetes, psoriasis, pemphigus

20   vulgaris, and rheumatoid arthritis. In particular, the therapeutic composition embodiment of the invention can be used to treat an autoimmune disease which is an arthritic condition. Further, the therapeutic composition embodiment of the invention can be used to treat an autoimmune disease which is a demyelinating disease. In yet another embodiment, the therapeutic composition embodiment of the invention can be used to

25   treat an autoimmune disease which is an inflammatory disease. For example, an embodiment of the invention is a therapeutic composition to treat the autoimmune disease rheumatoid arthritis. In another example, an embodiment of the invention is a therapeutic composition to treat the autoimmune disease multiple sclerosis.

In another embodiment of the invention, a method is provided for obtaining an

30   MHC class II amino acid binding motif sequence in a mixture of synthetic peptide heteropolymers having therapeutic activity in a subject, comprising the steps of : (a) binding the mixture of synthetic heteropolymers to MHC class II protein molecules to

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form a heteropolymer-MHC protein complexes: (b) removing by peptidase enzyme digestion the amino terminal amino acid residues of the heteropolymers protruding from the heteropolymer-MHC protein complex to align amino termini of the heteropolymers to the edge of the MHC protein complexes: and (c) eluting the aligned heteropolymers from
5   the MHC protein by dissociating the complexes to release the amino terminal aligned heteropolymers having the binding motif. In this method an additional step (d) can comprise: determining the amino terminal sequence of the aligned heteropolymers to obtain the binding motif. Further, in this method an additional step (e) comprises: comparing the amino terminal sequence of the aligned heteropolymers to the
10   amino acid sequence of the synthetic heteropolymer composition. In this method, the MHC class II protein is associated with an autoimmune disease, for example, the autoimmune disease is an arthritic condition or a demyelinating condition.

In another embodiment of this method, an additional step (e) can comprise: synthesizing a plurality of peptide preparations, each peptide preparation having an amino
15   acid sequence of a binding motif. In a further aspect of this method, an additional step (f) comprises: determining the affinity of each of the synthesized peptides for the MHC class II protein.

Detailed Description of Specific Embodiments

Unless the context otherwise requires, as used in this description and in the
20   following claims, the terms below shall have the meanings as set forth:

The term "autoimmune condition" means a disease state caused by an inappropriate immune response that is directed to a self-encoded entity which is known as an autoantigen.

The term "derivative" of an amino acid means a chemically related form of that
25   amino acid having an additional substituent, for example, N-carboxyanhydride group, a γ-benzyl group, an ε,N-trifluoroacetyl group, or a halide group attached to an atom of the amino acid.

The term "analog" means a chemically related form of that amino acid having a different configuration, for example, an isomer, or a D-configuration rather than an L-
30   configuration, or an organic molecule with the approximate size and shape of the amino acid, or an amino acid with modification to the atoms that are involved in the peptide bond, so as to be protease resistant when polymerized in a peptide or polypeptide.

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The phrases "amino acid" and "amino acid sequence" can include one or more components which are amino acid derivatives and/or amino acid analogs comprising part or the entirety of the residues for any one or more of the 20 naturally occurring amino acids indicated by that sequence. For example in an amino acid sequence having one or

5   more tyrosine residues, a portion of one or more of those residues can be substituted with homotyrosine. Further, an amino acid sequence having one or more non-peptide or peptidomimetic bonds between two adjacent residues, is included within this definition.

The term "hydrophobic" amino acid means aliphatic amino acids alanine (A, or ala), glycine (G, or gly), isoleucine (I, or ile), leucine (L, or leu), proline (P, or pro), and valine

10   (V, or val), the terms in parentheses being the one letter and three letter standard code abbreviations for each amino acid, and aromatic amino acids tryptophan (W, or trp), phenylalanine (F, or phe), and tyrosine (Y, or tyr). These amino acids confer hydrophobicity as a function of the length of aliphatic and size of aromatic side chains, when found as residues within a protein.

15   The term "charged" amino acid means amino acids aspartic acid (D or asp), glutamic acid (E or glu), histidine (H or his), arginine (R or arg) and lysine (K or lys), which confer a positive (his, lys, and arg) or negative (asp, gly) charge at physiological values of pH in aqueous solutions on proteins containing these residues.

The term "anergy" means unresponsiveness of the immune system of a subject to an

20   antigen.

The term "subject" as used herein indicates a mammal.

The term "arthritic condition" means at least one symptom of rheumatoid arthritis found in at least a single joint of a subject having the condition, for example in a shoulder, knee, hip or a digit of the subject. Examples of arthritic conditions include "polyarthritis",

25   which is an arthritic condition that affects more than a single joint; "juvenile arthritis", an arthritic condition of a subject under the age of 21; and Felty's syndrome, which includes along with symptoms of rheumatoid arthritis (RA) also the symptoms of neutropenia, splenomegaly, weight loss, anemia, lymphadenopathy, and pigment spots on the skin.

The term "heterologous cell" means a cell for production of an MHC protein which

30   is unrelated to a cell of a subject, i.e., the heterologous cell is not a cell of a mammal. Preferably the heterologous cell is not from a warm blooded animal, even more preferably the heterologous cell is not from a vertebrate; in the most preferred embodiment the

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heterologous cell is an insect cell, or a cell of a
microorganism such as a yeast cell.

    The term "pharmaceutically acceptable carrier"
includes any and all solvents, dispersion media, coatings,
5 antimicrobials such as antibacterial and antifungal
agents, isotonic and absorption delaying agents and the
like that are physiologically compatible. Preferably, the
carrier is suitable for intravenous, intramuscular, oral,
intraperitoneal, transdermal, or subcutaneous
10 administration, and the active compound can be coated in a
material to protect it from inactivation by the action of
acids or other adverse natural conditions.

    This invention is directed to methods of use of a
class of agents that can bind to specific MHC class II
15 proteins. Such agent can bind to a class II protein, and
thus inhibit and/or prevent the binding of an autoantigen
involved in an autoimmune disease, or upon binding can
induce anergy, so that there is no response of the immune
system to the autoantigen.

20   The class II MHC protein consists of two
approximately equal-sized subunits, α and β, which are
transmembrane proteins. A peptide-binding cleft, which is
formed by protein features from the amino termini of both
α and β subunits, is the site of presentation of the
25 antigen to T cells. There are at least three types of
class II MHC molecules: HLA-DR, -DQ, and -DP, and there
are numerous alleles of each type. The class II MHC
molecules are expressed predominantly on the surfaces of B
lymphocytes and antigen presenting cells such as
30 macrophages (Mengle-Gaw, L., *The Major Histocompatibility
Complex* (MHC), in the Encyclopedia of Molecular Biology,
Oxford: Blackwell Science Ltd., 1994, pp. 602-606).

    A composition of the present invention can be
administered by a variety of other methods known in the
35 art as will be appreciated by the skilled artisan. The

active compound can be prepared with carriers that will
protect it against rapid release, such as a controlled
release formulation, including implants, transdermal
patches, microencapsulated delivery systems. Many methods
5   for the preparation of such formulations are patented and
are generally known to those skilled in the art. See,
e.g., *Sustained and Controlled Release Drug Delivery
Systems*, J.R. Robinson, Ed., Marcel Dekker, Inc., NY,
1978. Therapeutic compositions for delivery in a
10 pharmaceutically acceptable carrier are sterile, and are
preferably stable under the conditions of manufacture and
storage. The composition can be formulated as a solution,
microemulsion, liposome, or other ordered structure
suitable to high drug concentration.
15   Dosage regimens can be adjusted to provide the
optimum desired response (e.g., a therapeutic response).
For example, a single bolus can be administered, several
divided doses can be administered over time, or the dose
can be proportionally reduced or increased as indicated by
20 the exigencies of the disease situation.
        In general, a preferred embodiment of the invention
is to administer a suitable daily dose of a therapeutic
composition that will be the lowest effective dose to
produce a therapeutic effect, for example, mitigation of
25 symptoms. The therapeutic compounds of the invention are
preferably administered at a dose per subject per day of
at least 2 mg, at least 5 mg, at least 10 mg or at least
20 mg as appropriate minimal starting dosages. In
general, the compound of the effective dose of the
30 composition of the invention can be administered in the
range of 50 - 400 micrograms of the compound per kilogram
of the subject per day.
        A physician or veterinarian having ordinary skill in
the art can readily determine and prescribe the effective
35 dose of the pharmaceutical composition required. For

example, the physician or veterinarian could start doses
of the compound of the invention employed in the
pharmaceutical composition at a level lower than that
required in order to achieve the desired therapeutic
5   effect, and increase the dosage with time until the
desired effect is achieved.

In another preferred embodiment, the pharmaceutical
composition includes also an additional therapeutic agent.
Thus in a method of the invention the pharmaceutical
10   composition can be administered as part of a combination
therapy, i.e. in combination with an additional agent or
agents. Examples of materials that can be used as
combination therapeutics with the peptides of the
invention for treatment of autoimmune disease and
15   arthritic conditions as additional therapeutic agents
include: an antibody or an antibody fragment that can bind
specifically to an inflammatory molecule or an unwanted
cytokine such as interleukin-6, interleukin-8, granulocyte
macrophage colony stimulating factor, and tumor necrosis
20   factor-α; an enzyme inhibitor which can be a protein, such
as α_1-antitrypsin, or aprotinin; an enzyme inhibitor which
can be a cyclooxygenase inhibitor; an engineered binding
protein, for example, an engineered protein that is a
protease inhibitor such as an engineered inhibitor of
25   kallikrein; an antibacterial agent, which can be an
antibiotic such as amoxicillin, rifampicin, erythromycin;
an antiviral agent, which can be a low molecular weight
chemical, such as acyclovir; a steroid, for example a
corticosteroid, or a sex steroid such as progesterone; a
30   non-steroidal anti-inflammatory agent such as aspirin,
ibuprofen, or acetaminophen; an anti-cancer agent such as
methotrexate or adriamycin; or a cytokine. An additional
therapeutic agent can be a cytokie, which as used herein
includes without limitation agents which are naturally
35   occurring proteins or variants and which function as

growth factors, lymphokines, interferons, tumor necrosis
factors, angiogenic or antiangiogenic factors,
erythropoietins, thrombopoietins, interleukins, maturation
factors, chemotactic proteins, or the like. Preferred
5 combination therapeutic agents to be used with the
composition of the invention and which are cytokines
include interleukin-4 and interleukin-10. A therapeutic
agent to be used with the composition of the invention can
be an engineered binding protein, known to one of skill in
10 the art of remodeling a protein that is covalently
attached to a virion coat protein by virtue of genetic
fusion (Ladner, R. et al., U.S. Patent 5,233,409;
Ladner,R. et al., U.S. Patent 5,403,484), and can be made
according to methods known in the art. A protein that
15 binds any of a variety of other targets can be engineered
and used in the present invention as a therapeutic agent
in combination with a peptide of the invention.

An improvement in the symptoms as a result of such
administration is noted by a reduction in edema of one or
20 more joints, by a reduction in inflammation in one or more
joints, or by an increase in mobility in one or more
joints. A therapeutically effective dosage preferably
reduces joint inflammation and edema and improves mobility
by at least about 20%, more preferably by at least about
25 40%, even more preferably by at least about 60%, and even
still more preferably by at least about 80%, relative to
untreated subjects.

The therapeutic compounds of the ivnention can be
used to treat symptoms of an autoimmune disease, a class
30 of disorder which include Hashimoto's thyroiditis;
idiopathic myxedema, a sever hypothyroidism; multiple
sclerosis, a demyelinating disease marked by patches or
hardened tissue in the brain or the spinal cord;
myasthenia gravis which is a disease having progressive
35 weakness of muscles caused by autoimmune attack on
acetylcholine

receptors at neuromuscular junctions; Guillain-Barre
syndrome, a polyneuritis; systemic lupus erythematosis;
uveitis; autoimmune oophoritis; chronic immune
thrombocytopenic purpura; colitis; diabetes; Grave's
5   disease, which is a form of hypothyroidism; psoriasis;
pemphigus vulgaris; and rheumatoid arthritis (RA).

Another embodiment of the invention is a kit for
assaying the binding of an analyte to an MHC protein.
This embodiment provides: a water-soluble MHC protein
10   which has been recombinantly produced in a non-mammalian
cell; a reaction chamber for containing the analyte and
the MHC protein; and means for detecting binding of the
analyte to the MHC protein. In a preferred embodiment,
the MHC protein is produced in an invertebrate or a
15   microbial cell, such as an insect cell or a yeast cell,
and so is devoid of bound peptide in the antigen cleft,
i.e., the MHC protein is "empty". Means for detecting
binding of the analyte to the MHC protein can be
radioactive, fluorimetric, chemiluminescent, or
20   colorimetric means known to one of ordinary skill in the
art. In a preferred embodiment of the kit, the MHC
protein is a class II MHC HLA-DR1 or -DR4 protein.
Further, the kit can include also an autoantigenic
peptide, such as a CII peptide, or a peptide derived from
25   myelin basic protein, myelin oligodendrite protein, or a
peptide from some other protein implicated in an
autoimmune disease.

Previous findings suggested that the activity of YEAK
(Cop 1) in EAE and MS involves binding to class II MHC
30   molecules within the peptide binding groove, resulting in
suppression of autoimmune T cell responses that can be
related to MS (Teitelbaum, D., et al., 1988, *Proc. Natl.
Acad. Sci.* USA 85:9724; Teitelbaum, D., et al., 1992,
Proc. Natl. Acad. Sci. USA 89:137; Fridkis-Hareli, M. et
35   al., 1998, *J. Immunol.* 160:4386, the contents of which are
hereby incorporated by reference).

The recombinant empty HLA-DR1 and -DR4 molecules that were here used in assays yielded data for binding that is free from interference due to previously bound endogenous peptides. In contrast, for prior analyses of binding to
5 human HLA-DR1 and -DR4 molecules, only 10-20% of the receptor proteins had been available for binding of exogenously supplied peptide (see, for example, Hammer, J. et al., 1993, *Cell* 74:197-203), resulting in determinations of binding affinities for the peptides that
10 in the present invention are different and more accurate compared to those reports.

YEAK binds with high affinity and in a peptide-specific manner to purified MS-associated HLA-DR2 (DRB1*1501) and rheumatoid arthritis (RA)-associated HLA-
15 DR1 (DRB1*0101) or HLA-DR4 (DRB1*0401) molecules. Since YEAK is a mixture of random polypeptides, it may contain different sequences that bind to different HLA proteins; in this case only a fraction out of the whole mixture would be an "active component". Alternatively, the whole
20 mixture may be competent, i.e. all polypeptides binding to any HLA-DR molecule. Example 1 shows methods for isolating and purifying a fraction of YEAK that bound to recombinant "empty" HLA-DR1, -DR2 and -DR4 molecules, produced so as to have minimal interference from
25 endogenous human peptides. Example 2 shows the distribution of amino acid residues in the fraction of YEAK molecules that bound to the HLA-DR protein molecules. The amino acid composition, HPLC profiles and pool sequence, and immunological recognition of the fraction of
30 the heteropolymer bound to MHC class II protein groove were determined.

Since the average length of the YEAK polypeptides used was 75-80 amino acids, the amino acid sequences comparable to "epitopes" lying in the groove of HLA-DR
35 molecules were likely to be found internally within the

polypeptide chains. The presence of the contiguous amino
ends of the polymer that were protruding from the
complexes could obscure the sequences of binding motifs to
be obtained by microchemical methods of sequence analysis
5   applied directly to the bound YEAK fraction. Because of
this consideration, amino-terminal aminopeptidase
treatment in Example 3 of the protruding ends of YEAK
polypeptides was employed to access the internal regions
and obtain the binding motif sequences. Since the
10 aminopeptidase trims amino-terminal ends of peptides that
protrude from the Class II MHC proteins, epitopes that
were bound to the groove of the proteins can be protected
from aminopeptidase proteolysis.

In Example 4, various 15-mer amino acid peptides were
15 synthesized to resemble sequences of the MHC class II DR-1
and -4 binding motifis obtained from the binding motif
sequences found in Example 3. The peptides were tested in
Example 5 to determine if they differentially inhibited
binding of disease-associated HLA-DR1 (DRB1*0101) or HLA-
20 DR4 (DRB1*0401) protein molecules to YEAK and to the
immunodominant epitope of collagen type II (CII) 261-273,
a candidate autoantigen in rheumatiod arthritis (RA).
Peptide sequences in Example 6 were further tested to
obtain those with ability to inhibit significantly the
25 response of HLA-DR1-and-DR4-restricted T cell clones to
the CII epitope 261-273 in cell culture *in vivo*. The
findings that certain peptides bind with high specificity
and affinity and inhibit T cell activation in Examples 9
and 10 indicate utility of certain of the 15-mer amino
30 acid peptide compounds as therapeutic agents in treatment
of autoimmune diseases such as RA and MS.

35

EXAMPLES

Methods for preparing reagents
Synthesis of heteropolymers and peptides

5

Heteropolymer YEAK (Cop 1) was prepared as described
by polymerization of the N-carboxyanhydrides of L-alanine,
γ-benzyl-L-glutamate, ε, N-trifluroacetyl-L-lysine, and L-
tyrosine (Teitelbaum, D., et al., 1971, *Eur. J. Immunol.*
10   1:242). The end product is a mixture of acetate salts of
random polypeptides. Heteropolymers EAK, batch SD-1689,
MW 8,850; YEA, batch SD-1690, MW 7,600; YAK, batch SD-
1691, MW 20,000; and YEK, batch SD-1697, MW 11,050 were
synthesized also by polymerization of the N-
15   carboxyanhydride substrate (Teva Pharmacuticals, Petch
Tieva, Israel; Fridkis-Hareli, M. et al., 1998, *J.
Immunol.* 160:4386, the contents of which are hereby
incorporated herein by reference). Heteropolymers can be
synthesized also by solid state techniques. Natural
20   peptide sequences influenza hemagglutinin HA peptide 306-
318 having the sequence PKYVKQNTLKLAT(SEQ ID NO: 1) and
collegan II(CII) peptide 261-273 having the sequence
AGFKGEQGPKGEP (SEQ ID NO:2) were synthesized using solid
phase techniques (Barany, G. et al., 1979, *Academic Press*,
25   New York. P.1) on an Applied Biosystems Peptide
Synthesizer (Foster City, CA) and purified by reverse-
phase HPLC. For these and other methods used throughout
these examples, see also Fridkis-Hareli et al., 1998,
Proc. Natl. Acad. Sci. U.S. 95:12528-12531, Fridkis-Hareli
30   et al., 1999 J. Immunol. 162:4697-4704, and Fridkis-Hareli
et al., 1999, Internet. Immunol. 11:635-641, the contents
of each of which are herein incorporated by reference
hereby.

The one letter and the three letter amino acid codes
35   (and the amino acid that each represents) are as follows:
A means ala (alanine); C means cys (cysteine); D means asp
(aspartic acid); E means glu (glutamic acid); F means phe

(phenylanine); G means gly (glycine); H means his
(histidine); I means ile (isoleucine); K means lys
(lysine); L means leu (Leucine); M means met (methionine);
N means asn (asparagine); P means pro (proline); Q means
5 gln (glutamine); R means arg (arginine); S means ser
(serine); T means thr (threonine); V means val (Valine); W
means trp (tryptophan); and Y means tyr (tyrosine).

Protein expression and purification
10

Recombinant HLA-DR1 and -DR4 molecules were expressed
in *Drosophila* S2 cells as described (Stem, L. et al.,
1992, *Cell* 68:465; Dessen, A. et al. 1997, *Immunity*
7:473). Cells were grown in roller bottles at 26°C in
15 Excell 401 medium (Sigma, St. Louis. MO) supplemented with
0-5% fetal bovine serum (Sigma). Cells were induced by
addition of CuSO$_4$ to 1 mM final concentration, and cells
were incubated an additional 4-5 days. Immunoaffinity
purification of recombinant HLA-DR1 and -DR4 was performed
20 as previously reported (Stern, L. et al., 1992, *Cell*
68:465; Dressen, A. et al., 1997, *Immunity* 7:473).
Supernatant from harvested cells was sequentially passed
through Protein A, Protein G and Protein A-LB3.1 columns,
followed by elution of the bound HLA-DR with 50mM 3-
25 cyclohexylamino-1 propane sulfonic acid (CAPS), pH 11.5,
and neutralized with 200 mM phosphate (pH 6.0). The
eluate was concentrated on a Centriprep 10 membrane
(Amicon). Protein concentrations were determined by
bicinchoninic acid assay (Pierce Chemical CO.).
30

Class II-peptide-binding assays

The solutions used in this assay are described in
Fridkis-Hareli, M. et al. 1998, *J Immunol.* 160:4386.
35 Assays were performed in 96- well microtitier immunoassay
plates (PRO-BIND™, Falcon) which were coated with
affinity-purified LB3.1 monoclonal antibodies, 100 µl of

1.0 µg/well in PBS (150 mM sodium chloride, 7.5 mM sodium phosphate dibasic, 2.5 mM sodium phosphate monobasic, pH 7.2) by incubation for 18 hrs at 4°C. The wells were then blocked with TBS (137 mM sodium chloride, 25 mM TRIS pH

5 8.0, 2.7 mM potassium chloride) containing 3% BSA (bovine serum albumin) for 1 hr at 37° and washed three times with TTBS (TBS with 0.05% Tween-20). Before sample addition, 50µl of TBS containing 1% BSA was added to each well.

Water-soluble HLA-DR1 molecules were recombinantly
10 produced in a heterologous host cell, for example, insect cells infected with recombinant baculoviruses (Stern, L.J. et al., 1992, *Cell* 68:465), specifically in *Drosphila* S2 cells as described supra. Binding analysis was performed by coincubating biotinylated YEAK, YEA, YAK, EAK or YEK
15 (final concentration, 1.5 µM) in 50 µl of the binding buffer in duplicate with varying concentrations of unlabeled inhibitors (YEA, YAK, EAK, YEK, YEAK, CII 261-273 or HA 306-318, and with recombinant water soluable DR molecules (0.15µM) for 40 hr at 37°C at pH5.0.

20

Detection of peptide-class II complexes

Bound peptide-biotin was detected using streptavidin-conjugated alkaline phosphatase as follows. Plates were
25 washed three times with TTBS and incubated with 100µl of streptavidin-conjugated alkaline phosphatase (1:3000, BioRad, Richmond, VA) for 1 hr at 37°C, followed by addition of *p*-nitrophenyl phosphate in triethanolamine buffer (BioRad). The absorbance at 410nm was monitored by
30 a microplate reader (model MR4000, Dynatech, Chantilly, VA).

T cell hybridomas and antigen presentation assays

35 The following mouse T cell hybridomas specific for CII were used: DR1-restricted 3.19 and 19.3 clones (Rosloniec, E.F., et al., 1997, *J. Exp. Med.* 185:1113-

1122), and DR4-restricted 3838 and D3 clones (Andersson, E.C., et al., 1998, *Proc. Natl. Acad. Sci.* USA). APC were L57.23 (L cells transfected with DR1 (Rosloneic, E.F. et al., 1997, *J. Exp. Med.* 185:1113-1122)), L Cells

5　transfected with DR4, and Priess cells (DRB1*0401/DRB4*0101). T cell stimulation experiments were performed in 96-well microtiter plates in a total volume of 0.2ml. Irradiated (3000rad) APC (2.5 x 10^4/well) were coincubated with CII 261-273 (40 μg/ml) and varying

10　concentrations of heteropolymers or peptides for 2 hr at 37°C, then T cells (5 x 10^4/well) were added and incubations were continued for 24 hr at 37°C. Supernatants (30 μl) wre removed and incubated with IL-2-dependent CTL-L (5 x 10^4/well) for 12 hr, followed by labeling with ^{3}H-

15　thymidine (1 μCi/well) for 12 hr. Plates were harvested and the radioactivity was monitored using a 1450 microbeta Plus liquid scintillation counter (Wallac, Gaithersburg, MD).

Example 1. *Methods for preparation and quantitation of*

20　*YEAK bound to HLA-DR1, -DR2 and -DR4 molecules.*

YEAK was incubated with water-soluble HLA-DR1, -DR2 or -DR4 molecules at the molar ratio of 1:1 for 40 hr at 37°C. These recombinant "empty" HLA-DR molecules can be stably assembled in the presence of exogenously added

25　antigen, and YEAK can function to promote stabilisation and with no interference from endogenous peptides (Fridkis-Hareli, M. et al., 1998. *J. Immunol.* 160:4386). Unbound YEAK was separated from bound YEAK by Centricon ultrafiltration. Bound YEAK was then extracted from the

30　HLA-DR complex by acid treatment (Chicz, R. et al., 1993. *J. Exp. Med.* 178:27) and subjected to amino acid analysis.

For HPLC separation and microsequencing after elution, approximately 5-10% of the YEAK mixtures were fractionated by microbore HPLC using a Zorbax C_{18} 1.0mm

35　reverse-phase column on a Hewlett-Packard 1090 HPLC with 1040 diode array detector.

Pages 21 to 25 have been intentionally left blank.

5

At a flow rate of 54 μl/min, YEAK was eluted with a gradient of 0.055% trifluoroacetic

acid (TFA) in acetonitrile (0% at 0 to 10 min, 33% at 73 min and 60% at 105 min).

Strategies for peak election, reverse phase separation and Edman microsequencing were

performed as in Chicz, R. et al. 1993. *J. Exp. Med. 178:27*, and Lane, W. et al. 1991. *J.*

5   *Prot. Chem 10:151.*

To further characterize the bound fraction of YEAK by means of hydrophobicity

and size, samples were separated on RP-HPLC using an acetonitrile gradient. Untreated

YEAK showed a very broad peak with several smaller peaks, which spread between

approximately 40 and 75 min elution time. This elution profile is characteristic of a

10   mixture of random polypeptides and resembles HPLC separations of other batches of

YEAK. Similar profiles were obtained when YEAK was eluted from HLA-DR1, -DR2 or

-DR4 molecules, indicating that the bound fraction is similar to the whole original YEAK

mixture in its chemical properties.

Example 2.   Analysis of YEAK bound to HLA-DR1, -DR2, and -DR4 molecules.

15   At least 95% of the added YEAK heteropolymer molecules was observed in the

fraction that was bound to isolated HLA-DR1 and HLA-DR4, and 80% was bound to

HLA-DR2 proteins. YEAK that was eluted from the complexes with HLA-DR1, -DR2

and -DR4 molecules showed ratios of the component amino acids Y:E:A:K similar to that

of control untreated YEAK. These results indicate that the bound fraction of YEAK

20   reflected the amino acid composition of the whole mixture and that the YEAK population

exhibited little or no preferential binding to different HLA-DR proteins. When YEAK

was incubated with an excess of each of HLA-DR1, -DR2 and -DR4 molecules that had

been purified from human homozygous EBV-transformed B cell lines, and the complexes

were further fractionated by passage through a size-exclusion column, the distribution of

25   eluted material showed that nearly all of the YEAK was found in the fractions

corresponding to the high molecular weight complexes, with less than 10% at the lower

molecular weight position of control YEAK, for each of the HLA-DR molecules.

To analyze the sequence of YEAK that bound to each of HLA-DR1, -DR2 and -

DR4 molecules, HPLC fractions obtained in Example 5 were pooled within the areas of

30   elution, and pooled fractions were submitted to automated Edman degradation on a

Hewlett-Packard G1005A (Palo Alto, CA) protein sequencer using the manufacturer's

Routine 3.5.

-26-

For each of the HLA-DR proteins, the results showed that the four amino acid components of YEAK bound to protein were randomly distributed within the sequence according to the input molar ratios of YEAK. Amino acid alanine (A) was found at significantly higher levels compared to E, Y and K, as expected from the initially higher
5 molar ratio of A in YEAK. There was no sequence specificity or preferential positioning of any of the amino acids of YEAK, indicating that the bound fraction was also random and similar to the entire unfractionated YEAK.

Anti-YEAK polyclonal antibodies were used to determine whether fractions of YEAK eluted from each of the HLA-DR molecules contained the epitopes found in
10 control untreated YEAK. The cross reactivity between YEAK and various YEAK ∿ fractions was detected by direct ELISA assay using biotinylated anti-YEAK polyclonal antibodies. YEAK or fractions were diluted to 0.4 μg/ml and 2.0 μg/ml and 100 μl/well was plated in duplicate on a 96-well microtiter immunoassay plate (PRO-BIND™, Falcon, Lincoln Park, NJ), incubated for 1 hr at 37°C and washed three times with TBS
15 containing 0.05% Tween-20. The wells were then blocked with TBS containing 3% BSA, followed by addition of biotinylated anti-YEAK antibodies (at a dilution of 1:5000, 100 μl/well). Antibody-ligand complexes were detected using streptavidin-conjugated alkaline phosphatase (at a dilution of 1:3000, BioRad) and p-nitrophenyl phosphate in triethanolamine buffer (BioRad; Hercules, CA). The absorbance at 410 nm was
20 monitored by a microplate reader (Dynatech MR4000).

The antibody binding assays showed that all the fractions were similarly recognized by anti-YEAK antibodies, suggesting that these bound heteropolymer fractions shared similar or identical epitopes with each other and with control YEAK.

Example 3. Characterization of binding motifs of YEAK by removal of protruding amino
25 *termini of YEAK bound to HLA-DR1, -DR2 or -DR4 molecules with aminopeptidase I.*

The sequences of the first 20 to 25 N-termini amino acids observed in Example 6 2 represent the sequences that protrude from beyond the HLA-DR molecules, so are not a source of information regarding the actual binding motif(s) of YEAK bound within the functional epitope-specific groove. To obtain the amino acid sequence of the portion of
30 the YEAK molecule bound within the MHC class II protein and so protected by this protein, YEAK (1mM) was initially incubated with each of the HLA-DR molecules (100 μm) in a volume of 10 μl at the molar ratio of 10 YEAK:1 HLA-DR, in PBS for 40 hours

-27-

at 37°C. Aminopeptidase I, a metalloprotein isolated from *Streptomyces griseus* (Spungin

A. et al. 1989. *J. Biochem. 183:471*; available from Sigma Chemicals, St. Louis, MO),

was added to the reaction in a volume of 2 μl containing 2 units for the last 18 hr of

incubation, in order to remove amino-terminal ends of YEAK polypeptides protruding

5   from the HLA-DR molecules, and to digest remaining unbound YEAK (Mouritsen, S. et

al. 1992. *J. Immunol. 148:1987*; Larsen, S. L. et al. 1996. *J. Exp. Med. 184:183*).

Subsequent digestions of heteropolymer with aminopeptidase was performed in volumes

scaled up by a factor of twenty-forty fold, for example, 300 μl of heteropolymer digested

with 60 μl of aminopeptidase. Samples were spin-concentrated to a final volume of

10   approximately 100 μl using Centricon 10 ultrafiltration devices.

The YEAK-HLA-DR complexes and the unbound YEAK were analyzed by SDS-

PAGE. SDS-PAGE was carried out with the NOVEX mini cell electrophoresis system.

Separation gel was 10% in acrylamide and stacking gel was 5%. HLA-DR1-YEAK

complexes were run under nonreducing conditions for 1 hr at 200 V, stained with

15   Coomassie Brilliant Blue, fixed for 3 hr in 10% methanol/10% acetic acid and dried on

Cellophane paper (BioRad) at 25°C. The YEAK-HLA-DR complexes were found to be

resistant to SDS-induced dissociation, forming higher molecular weight complexes with

HLA-DR1 αβ heterodimers, and were observed as numerous bands on the polyacrylamide

gel with molecular weights greater than the molecular weight protein standard of 50 kD,

20   showing that the YEAK-DR complexes were protected. Aminopeptidase I treatment

resulted in unbound YEAK appearing as a smear in the lower part of the gel, showing that

it was completely digested by the enzyme.

To obtain the sequence of the binding motifs, fractions containing the peaks of

protected YEAK were selected in the region between approximately 40 and 75 min

25   elution time for each class of HLA-DR complex. Bound YEAK absent the protruding N-

termini was eluted from HLA-DR by addition of acetic acid (10%) and incubation at 70°C

for 15 min, followed by ultrafiltration and vacuum concentration in a SpeedVac (Savant

Instruments, Farmingdale, NY; Fridkis-Hareli, M. et al. 1995. *Cell. Immunol. 163:229*).

The sequence data (Table 2) show that for peptides bound to HLA-DR1, significantly

30   higher levels of the E residue were found at the first and second cycles, higher levels of K

residue were found at the second and third cycles, and higher levels of Y residue were

found at the third to fifth cycle (presumably at the position corresponding approximately

-28-

to the P1 of the bound peptide site within the MHC class II groove). The amino acid
residue obtained from position 3 from the Edman degradation method corresponds to the
P1 anchor position of the MHC class II peptide binding groove, since in the structure of
the HA 306-318 complex with HLA-DR1, the P-2 amino acid residue is at the flush end
5   of the groove and the P1 position is the third amino acid, that is, Y308, in a deep pocket
(Stern, L. et al., *Nature (Lond.) 368:215*). These data are in contrast to the random
patterns of the sequences found in untreated YEAK, which showed no sequence
specificity or preferential positioning within the MHC class II groove of any of the four
amino acids that comprise YEAK.

10   For HLA-DR2, both Y and A residue levels were enriched at cycle 3 (Table 1). No
sequence specificity or preferential positioning was observed for positions corresponding
to anchor positions following P1 (at positions in the sequence that correspond to the P4,
P6 or P9 of HLA-DR1 or -DR4; P4, P7 of DR2b molecules). In all the samples the levels
of A were higher than those of E, Y and K, a finding which was expected and corresponds
15 to the higher molar ratio of A in YEAK. For each of the HLA-DR-1 and -4 molecules, Y
was found at the position corresponding to the first anchor position (the third residue in
the sequence analysis), followed by A in the positions corresponding to the subsequent
pockets. In the YEAK bound to HLA-DR2 also, Y was enriched at the position
corresponding to P1. At the first cycle position corresponding to the P-2 position, E was
20 enriched, and at the next adjacent position corresponding to P-1, K was enriched. These
residues can contribute to the stable interactions of YEAK with the HLA-DR molecules
and the interaction of this complex with the T cell receptor (TCR).

           These results indicate that YEAK contains class II MHC binding motifs. Without
being bound by any particular theory, it is shown by these data that YEAK, bound to the
25 antigen groove of HLA-DR molecules, can act either as a blocking peptide or as an
antagonist or partial agonist, resulting in suppression of autoimmune T cell responses or
anergy, or both. The binding motif sequences are useful for mapping the T cell epitopes,
and for design of novel agents for the treatment of autoimmune diseases, such as MS and
RA in humans.

30   *Example 4: Synthesis of peptides having binding motifs for HLA-DR1 and -DR4
molecules*

           Examples above show that the YEAK heteropolymer bound to purified human

-29-

HLA-DR molecules within the peptide binding groove and inhibited the binding of HA 306-318

Table 1. Binding motif sequences of YEAK bound to HLA-DR1, -DR2 and -DR4
molecules

HLA-DR		relative amino acid positions						
		-2	-1	1	4	6	7	9
DRB1*0101	DR-1	E	K	Y	A	A	A	A
DRB1*0401	DR-4	E	K	Y	A	A	A	A
DRB1*1501	DR-2	E	K	Y,A	A	A	A	A

peptide, a high affinity epitope of influenza virus, to both HLA-DR1 (DRB1*0101) and -DR4 (DRB1*0401) molecules. Further, random heteropolymers composed of only three amino acids (EAK, YEA, YAK and YEK) bound to purified HLA-DR1, -DR2 and -DR4 molecules and competed with CII 261-273 for binding to RA-associated HLA-DR1 (DRB1*0101) and -DR4 (DRB1*0401) protein molecules, and inhibited CII-reactive T cell clones. The fraction of YEAK that bound to the protein was isolated from complexes with recombinant "empty" HLA-DR molecules produced in insect cells, and binding motifs were resolved by aminopeptidase I treatment of the YEAK that bound to the complex in the major groove of HLA-DR1 or -DR4 molecules. Subsequent pool sequencing of eluted peptides showed increased in levels of E at the first and second cycles, of K at the second and third cycles, and of Y (at P1 of the bound peptide) at the third to fifth cycle of the amino acid residues, regardless of the HLA-DR molecule employed.

In this Example, peptides of defined sequence and 15 residue length were synthesized using the sequences of the binding motifs summarized in Table 1. These peptides were analyzed in the Examples below for affinity and specificity of binding to MHC class II HLA DR protein molecules and for ability to inhibit binding of competitor molecules and ability to inhibit T cell responses, functional properties appropriate to a

-30-

novel therapeutic composition for an autoimmune disease.

Peptides shown in Table 2 were synthesized using solid phase techniques (Barany, G. et al., 1979. *The Peptides*, E. Gross et al., eds. (New York, NY: Academic Press) on an Applied Biosystems Peptide Synthesizer, and were purified by reversed-phase HPLC.

5   Peptide sequences included HA 306-318, PKYVKQNTLKLAT (SEQ ID NO: 1), MW 1718; CII 261-273, AGFKGEQGPKGEP (SEQ ID NO: 2), MW 1516; and HA 306-318 bracketed by alanines at N- and C-terminals, APKYVKQNTLKLATA (SEQ ID NO: 4). For comparison, the cII 261-273 peptide, bracketed by alanines at N- and C-terminals, AGFKGEQGPKGEP (SEQ ID NO: 3), can be synthesized. Peptides were also

10   synthesized on a 1 μmole scale using the Multipin Peptide Synthesis System (Chiron Technologies, Raleigh, NC). Peptides were synthesized as 15-mers with free amino groups at the N-terminus and free carboxyl groups at the C-terminus, and with biotin linked to the N-terminus by the spacer SGSG and having a free carboxyl group at the C-terminus. Peptide synthesis was monitored by including two standard peptide sequences

15   as controls, which were subjected to HPLC and mass spectroscopy analysis. HA 306-318 peptide was also used as a positive control for binding experiments. Pin peptides were lyophilized and resuspended at a concentration of 2 mg/ml in dimethyl sulfoxide (DMSO). Under these conditions, the majority of peptides were completely solubilized. Biotinylation was performed with excess N-hydroxysuccinimide biotin (Sigma, St.

20   Louis, MO) in DMSO as described (Fridkis-Hareli et al., 1994. *Proc. Natl. Acad. Sci., U.S.A.* 91:4872-4876). Unreacted biotin was removed by dialysis (Spectra/Por® membrane MWCO 500, Spectrum Medical Industries, Houston, TX).

The 15-mer peptides (SEQ ID NOs: 5-36; see Table 2) synthesized based on the motifs for binding of YEAK to the groove of HLA-DR1 and -DR4 molecules

25   contained various combinations of E, K and A at the N-terminus for most of the peptides, followed by Y at the position corresponding to P1 (shown in bold), and then A in the subsequent binding pockets. The sequences fall into three different groups according to these positions in the consensus (Table 2). Peptides in group I had K at the position corresponding to P8 and Y at the position corresponding to P1 (in bold in Table

30   2). A reference peptide in this set with lysine (K) at the position corresponding to P8 to increase solubility and alanine (A) at all other residues had previously been synthesized (SEQ ID NO: 5; Jardetzky, T. S., et al. 1990. *EMBO J. 9*, 1797-1803). Peptides in

-31-

group II had Y at the position corresponding to P1, however had A at the position corresponding to P8. Peptides in group III had amino acid tyrosine (Y) shifted one or two residues with respect to that in HA 306-318 peptide. Peptides in all groups contained one or more glutamic acid (E) and/or lysine (K) residues, as was observed in

5 the binding motifs supra, and to enhance solubility. Both N-terminal biotinylated and unlabeled sets of peptides were synthesized for these studies.

Table 2. Groups of synthetic peptides and consensus positions.

group	SEQ ID NO	peptide sequence	amino acid consensus positions
Control	4	APKYVKQNTLKLATA	A(HA 306-318)A
I.	5	AAAYAAAAAAKAAAA	P1Y, P8K
	6	AEKYAAAAAAKAAAA	
	7	AKEYAAAAAAKAAAA	
	8	AKKYAAAAAAKAAAA	
	9	AEAYAAAAAAKAAAA	
	10	KEAYAAAAAAKAAAA	
	11	AEEYAAAAAAKAAAA	
	12	AAEYAAAAAAKAAAA	
	13	EKAYAAAAAAKAAAA	
	14	AAKYEAAAAAKAAAA	
	15	AAKYAEAAAAKAAAA	
	16	EAAYAAAAAAKAAAA	
	17	EKKYAAAAAAKAAAA	
	18	EAKYAAAAAAKAAAA	
II.	19	AEKYAAAAAAAAAAA	P1Y, P8A
	20	AKEYAAAAAAAAAAA	
	21	AKKYEAAAAAAAAAA	
	22	AKKYAEAAAAAAAAA	
	23	AEAYKAAAAAAAAAA	
	24	KEAYAAAAAAAAAAA	
	25	AEEYKAAAAAAAAAA	
	26	AAEYKAAAAAAAAAA	
	27	EKAYAAAAAAAAAAA	
	28	AAKYEAAAAAAAAAA	
	29	AAKYAEAAAAAAAAA	
	30	EKKYAAAAAAAAAAA	
	31	EAKYAAAAAAAAAAA	
III.	32	AEYAKAAAAAAAAAA	P1A, P8A

33	AEKAYAAAAAAAAAA
34	EKYAAAAAAAAAAAA
35	AYKAEAAAAAAAAAA
36	AKYAEAAAAAAAAAA

5

Example 5. _Inhibition of YEAK and antigen binding to HLA-DR molecules by the synthetic 15-mer peptides_

10   To examine whether the synthetic peptides can compete successfully for binding to HLA-DR1 and -DR4 with YEAK or with the high affinity HA 306-318 peptide, competitive binding assays were carried out with both biotinylated YEAK or HA 306-318 (bracketed by alanines) and unlabeled inhibitors (YEAK and the synthetic 15-mer peptides). Kinetic studies indicated that biotinylated YEAK inhibited binding of unlabeled YEAK and

15   of HA 306-318 (peptide SEQ ID NO: 4) to recombinant HLA-DR1 better than of peptides in groups I-III. However, several peptides containing K at the position corresponding to P8 (group I) were better inhibitors than peptides that were similar but having A at the position corresponding to P8 (from groups II and III of Table 2). In contrast, the binding of biotinylated YEAK to HLA-DR4 molecules was efficiently inhibited by many of the

20   peptides in groups I-III, but the binding of biotinylated HA 306-318 to HLA-DR4 was better inhibited by YEAK than by HA 306-318 or by the 15-mer peptides.

       To further characterize the relative affinity of the synthetic 15-mer peptides to compete with each of YEAK, HA 306-318 or CII 261-273 for binding to HLA-DR1, -DR2 and -DR4 molecules, competitive binding assays were carried out with biotinylated

25   Multipin peptides and the three unlabeled inhibitors. The binding of the majority of the

Table 3 . Affinity of selected YEAK-related peptides for HLA-DR1 (DRB1*0101) molecules _determined by competition with biotinylated competitors HA306-318 and YEAK (μM)

30

| SEQ ID NO	peptide sequence	HA 306-318	YEAK
4	APKYVKQNTLKLATA	13.0	3.3
7	AKEYAAAAAAKAAAA	19.0	
12	AAEYAAAAAAKAAAA	47.0	
15	AAKYAEAAAAKAAAA	42.0	16.0
18	EAKYAAAAAAKAAAA	33.0	
	YEAK	10.0	8.0

35

40

peptides in groups I-III to both HLA-DR1 and HLA-DR4 was inhibited by unlabeled YEAK, HA 306-318 (SEQ ID NO: 4) or CII 261-273 (SEQ ID NO: 2), however, less efficiently than the binding of HA 306-318 (SEQ ID NO: 4). Some of the peptides however showed higher affinity for the HLA proteins than did YEAK, HA306-318, or

5   CII 261-273.

All peptides were further tested for ability to inhibit CII-specific T cell responses.

Example 6. Inhibition of HLA-DR1- and -DR4-restricted CII-specific T cell responses by the 15-mer synthetic peptides

To determine whether the synthetic peptides could also inhibit presentation of the
10   CII 261-273 peptide to autoreactive T cells, complexes of APC and peptides were tested with CII-specific T cell hybridomas restricted to HLA-DR1 (3.19 and 19.3) and HLA-DR4 (3838 and D3). Irradiated APC were incubated with CII 261-273 and of each of the relevant peptides for 2 hrs, T cells were added and the incubation continued for 24 hrs, and supernatants were tested to determine quantities
15   of IL-2 secretion by these hybridomas as a measure of T cell activation.

Peptides SEQ ID NOs: 15 and 26 were observed to be the most potent inhibitors of HLA-DR1-restricted T cells, using L fibroblasts transfected with HLA-DR1 as APC for the CII peptide. Peptides 15, 20, 26 and 27 inhibited responses to 19.3 T cells essentially 100%, to levels of inhibition greater than observed with HA 306-318. For
20   3.19 cells, inhibition by peptide #26 was equivalent to that of HA 306-318. YEAK had little effect on this CII-specific T cell response (inhibition less than 20%). HA 306-318 (peptide SEQ ID NO: 4) inhibited both DR1 3.19 and 19.3 T cell clones very efficiently (over 95% and 98% for 19.3 and 3.19 cells, respectively). These data show that peptides of SEQ ID NO: 15, 20, 26, and 27 were as good or better inhibitors of T cell response
25   than the reference influenza virus hemagglutinin peptide HA 306-318.

For HLA-DR4-restricted T cells, using L fibroblasts transfected with HLA-DR4 as APC, the following pattern of activity was obtained: peptides SEQ ID NOs: 6, 11, 16, 17, 22, 23, 27, 28 and 33 were good inhibitors of the DR4 3838 T cell clone, whereas the D3 clone was inhibited best by peptides SEQ ID NOs: 8, 15, 16, 18 and 27. These
30   peptides produced levels of inhibition of over 80% for the D3 and 3838 cells. YEAK had only a minimal effect on the CII-specific T cell response, consistently giving less than 20% inhibition. HA 306-318 (SEQ ID NO: 4) inhibited both DR4 3838 and D3 T
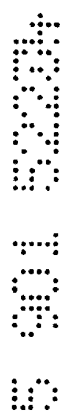
cell clones less efficiently (less than 60% inhibition) than it inhibited the DR1 3.19 and
19.3 clones. These data show that peptides of SEQ ID NO: 8, 15, 16, 18, and 27 were
significantly better inhibitors of T cell response than the reference influenza virus
hemagglutinin peptide HA 306-318. Peptides of SEQ ID NO: 15 and 27 were high
5 level inhibitors both of HLA-DR-1- and -DR-4-restricted CII-specific T cells.

Table 4. Affinity of selected YEAK-related peptides for HLA-DR4 (DRB1*0401)
molecules determined by competition with biotinylated competitors HA306-318 and
YEAK (μM)

10

SEQ ID NO	peptide sequence	HA 306-318	YEAK
4	APKYVKQNTLKLATA	26.0	8.2
5	AAAYAAAAAAKAAAA	7.0	
6	AEKYAAAAAAKAAAA	6.5	
7	AKEYAAAAAAKAAAA	4.5	
10	KEAYAAAAAAKAAAA	4.5	
11	AEEYAAAAAAKAAAA	2.0	
12	AAEYAAAAAAKAAAA	3.2	1.6
13	EKAYAAAAAAKAAAA	3.3	
14	AAKYEAAAAAKAAAA	4.0	
15	AAKYAEAAAAKAAAA	1.8	<1.0
16	EAAYAAAAAAKAAAA	5.0	
17	EKKYAAAAAAKAAAA	1.8	
18	EAKYAAAAAAKAAAA	4.4	3.0
21	AKKYEAAAAAAAAAA	2.2	
26	AAEYKAAAAAAAAAA	1.8	
28	AAKYEAAAAAAAAAA	1.2	
29	AAKYAEAAAAAAAAA	1.2	
32	AEYAKAAAAAAAAAA	3.0	
33	AEKAYAAAAAAAAAA	<1.0	
35	AYKAEAAAAAAAAAA	1.3	
36	AKYAEAAAAAAAAAA	3.0	
	YEAK	2.5	20.0

The data in these examples, performed with each peptide at least in duplicate, show
that of 32 unique synthetic peptides, several inhibited binding of HA 306-318 and YEAK
40 to recombinant HLA-DR1 and -DR4 molecules. Peptides which inhibited binding of HA
306-318 or YEAK to HLA-DR1 or -DR4 molecules contained Y at the P1 position. The
presence of E, A and K in various combinations on the N-terminal side of P1 did not seem

to influence the affinity of the binding. Of the subsequent residues, K at P8 was important for inhibition of HA 306-318 but not of YEAK binding to HLA-DR1. In contrast to HLA-DR1, a larger number of peptides inhibited binding of both HA 306-318 and YEAK to HLA-DR4 molecules. These peptides contained Y at the position corresponding to P1 and

5 either K or A at the position corresponding to P8, with no preferences for specific amino acids at other positions. The affinity of the HA 306-318 for recombinant HLA-DR4 was lower, and that of YEAK higher, than for HLA-DR1 molecules, similarly to the case observed with HLA-DR1 and -DR4 molecules purified human from blood. The binding of some of the biotinylated peptides to either HLA-DR1 or -DR4 was inhibited by CII 261-

10 273, as well as by HA 306-318 and YEAK, showing that these peptides may compete for presentation to CII-reactive T cells, similar to the whole YEAK mixture. Peptides with an affinity close to or higher than that of the reference natural peptides or the YEAK-mixture are listed in Tables 3 and 4 for HLA-DR1 and HLA-DR4, respectively.

Several of the 15-mer peptides inhibited type II collagen-specific T cell clones.

15 These peptides all had Y at the position corresponding to P1 and either K or A at the position corresponding to P8, with no other specific patterns. Examples here show the strong inhibition by several random heteropolymers composed of three amino acids selected from the group consisting of Y, E, A and K. These heteropolymers, especially YAK, competed with CII

20 261-273 for binding to RA-associated HLA-DR1 and -DR4 molecules, and inhibited CII-reactive T cell clones. Further, peptide SEQ ID NO: 8, which includes the direct sequence YAK, inhibited type II collagen-reactive T cells better than YEAK, indicating that a peptide of approximately 15 amino acids in length having the single sequence YAK can substitute for the mixture of random polypeptides found in the heteropolymer

25 poly(Y,A,K).

The results of the Examples that are the embodiments of the invention, that the individual components of Y, E, A and K or peptides have sequences that correspond to binding motifs for anchor positions fitting the particular HLA-DR molecule (Y at the position corresponding to P1) can act as effective therapeutic agents for autoimmune

30 diseases, substituting for a mixture of random polypeptides. A pharmaceutical composition comprising a pure synthetic short polypeptide of identified sequence can have fewer side effects when administered to a subject than a mixture of polypeptides
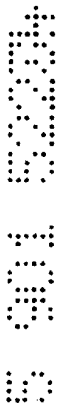
-37-

of random sequence. Further, a particular peptide sequences that is effective in binding

to an HLA-DR molecule can be embedded into a longer sequence, for example,

containing direct repeats of the peptide sequence or other molecules such as amino acid

analogs, to increase stability in vivo or to impart other desirable properties. A

5   pharmaceutical composition comprising a pure synthetic longer identified sequence can

be most effective in having greatest efficacy and least toxicity.

10

EDITORIAL NOTE

APPLICATION NUMBER - 52234/99

The following Sequence Listing pages 1 to 7 are part of the description. The claims pages follow on pages 38 to 43.

SEQUENCE LISTING

<110> Strominger, Jack L.
      Fridkis-Hareli, Masha

<120> SYNTHETIC PEPTIDES AND METHODS OF USE
  FOR AUTOIMMUNE DISEASE THERAPIES

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<150> 60/093,859
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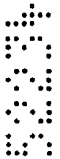
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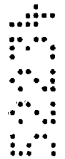
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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1.   An isolated peptide having a sequence selected from the group consisting of: AKEYAAAAAAKAAAA (SEQ ID NO: 7),
5   AAEYAAAAAAKAAAA (SEQ ID NO: 12), AAKYAEAAAAKAAAA (SEQ ID NO: 15), and EAKYAAAAAAKAAAA (SEQ ID NO: 18).

2.   An isolated peptide according to claim 1, in which the tyrosine (Y) has been substituted by a valine (V) or a
10   phenylalanine (F).

3.   A composition comprising an isolated peptide according to claim 1 or claim 2, and a pharmaceutically acceptable carrier.
15

4.   An isolated peptide having a sequence selected from the group consisting of: AEKYAAAAAAKAAAA (SEQ ID NO: 6), AKEYAAAAAAKAAAA (SEQ ID NO: 7), KEAYAAAAAAKAAAA (SEQ ID NO: 10), AEEYAAAAAAKAAAA (SEQ ID No: 11), AAEYAAAAAAKAAAA
20   (SEQ ID NO: 12), EKAYAAAAAAKAAAA (SEQ ID NO: 13), AAKYEAAAAAKAAAA (SEQ ID NO: 14), AAKYAEAAAAKAAAA (SEQ ID NO. 15), EAAYAAAAAAKAAAA (SEQ ID NO: 16), EKKYAAAAAAKAAAA (SEQ ID NO: 17), EAKYAAAAAAKAAAA (SEQ ID NO: 18), AKKYEAAAAAAAAAA (SEQ ID NO: 21), AAEYKAAAAAAAAA (SEQ ID
25   NO: 26), AAKYEAAAAAAAAA (SEQ ID NO: 28), AAKYAEAAAAAAAA (SEQ ID NO: 29), AEYAKAAAAAAAAA (SEQ ID NO: 32), AEKAYAAAAAAAAA (SEQ ID NO: 33), AYKAEAAAAAAAAA (SEQ ID NO: 35), and AKYAEAAAAAAAAA (SEQ ID NO: 36), the peptide having high affinity for an MHC class II protein.
30

5.   An isolated peptide according to claim 4, in which the tyrosine (Y) has been substituted by a valine (V) or a phenylalanine (F).

35   6.   A composition comprising an isolated peptide according to claim 4 or claim 5, and a pharmaceutically acceptable carrier.

7.   A pharmaceutical preparation comprising a first
peptide sequence and a second peptide sequence, wherein
the preparation is a mixture of a first peptide and a
5   second peptide of different amino acid sequences both
according to any one of claims 1 to 5, in a
pharmaceutically acceptable carrier.

8.   A pharmaceutical preparation according to claim 7,
10   wherein the first sequence has in addition a lysine
residue and the second sequence has in addition an alanine
residue, respectively, at the amino acid position
corresponding to eight residues beyond the amino acid
corresponding to the P1 pocket in the MHC class II peptide
15   binding groove.

9.   A method for obtaining an MHC class II binding motif
amino acid sequence in a mixture of synthetic
peptide heteropolymers having therapeutic activity in a
20   subject, comprising:
        (a) binding the mixture of synthetic heteropolymers
to MHC Class II protein molecules to form heteropolymer-
MHC protein complexes;
        (b) removing by peptidase enzyme digestion the amino
25   terminal amino acid residues of the heteropolymers
protruding from the heteropolymer-MHC protein complex to
align amino termini of the heteropolymers to the edge of
the MHC protein complexes; and
        (c) eluting the aligned heteropolymers from the MHC
30   protein by dissociating the complexes to release the amino
terminal aligned heteropolymers having the binding motif.

10.   A method according to claim 9, wherein an additional
step (d) comprises: determining the amino acid sequence of
35   the amino terminal of the aligned heteropolymers, to
identify the binding motif.

11.   A method according to claim 10, wherein an
additional step (e) comprises: comparing the amino acid
sequence of the amino terminal sequence of the aligned
heteropolymers to the amino acid sequence of the synthetic
5 heteropolymer composition.

12.   A method according to any one of claims 9 to 11,
wherein the MHC class II protein is associated with an
autoimmune disease.
10

13.   A method according to claim 12, wherein the
autoimmune disease is an arthritic condition or a
demyelinating condition.

15 14.   A method according to any one of claims 10 to 13,
wherein an additional step (e) or (f) comprises:
synthesizing a plurality of peptide preparations, each
peptide preparation having a binding motif amino acid
sequence.
20

15.   A method according to claim 14, wherein an
additional step (f) or (g) comprises: determining the
affinity of each of the synthesized peptides for the MHC
class II protein.
25

16.   A method for identifying an MHC class II amino acid
binding motif in a mixture of synthetic peptide
heteropolymer molecules, wherein the MHC class II is
associated with an autoimmune disease, the heteropolymer
30 molecules being a random sequence having a particular
molar ratio of component amino acids, comprising:
      (a) binding the mixture of synthetic heteropolymer
molecules to purified recombinant MHC class II protein
molecules to form heteropolymer-MHC protein complexes;
35   (b) removing by aminopeptidase enzyme digestion
amino terminal amino acid residues of heteropolymer
molecules protruding from the heteropolymer-MHC protein
complexes to produce resulting heteropolymers having

aligned amino termini and a consensus binding motif within
MHC class II protein complexes, the binding motif being
located at amino termini of the resulting heteropolymer
molecules;

5   (c) eluting the resulting aligned heteropolymer
molecules from the complexes by dissociating the
complexes; and

          (d) determining the amino acid sequence from the
amino terminal of the eluted aligned heteropolymer
10   molecules, to identify the binding motif.

17.   A method according to claim 16, wherein an
additional step (e) comprises: comparing the amino acid
sequence of the eluted aligned heteropolymer molecules to
15   the amino acid sequence of the synthetic heteropolymer
composition.

18.   A method according to claim 16 or claim 17, wherein
the autoimmune disease is an arthritic condition.
20

19.   A method according to claim 18, wherein the
arthritic condition is rheumatoid arthritis.

20.   A method according to claim 16 or claim 17, wherein
·25   the autoimmune disease is a demyelinating condition.

21.   A method according to claim 20, wherein the
demyelinating condition is multiple sclerosis.

30   22.   A method according to any one of claims 17 to 21,
wherein the MHC class II is selected from the group of DR1
and DR4.

23.   A method according to any one of claims 16 to 21,
35   wherein the MHC class II is DR2.

24.   A method according to any one of claims 16 or 18 to
23, wherein an additional step (e) or (f) comprises:
synthesizing a plurality of peptide compositions, each

peptide composition having an amino acid sequence
containing the binding motif.

25.   A method according to claim 24, wherein an
additional step (f) or (g) comprises: determining the
affinity of each of the synthesized peptides for the MHC
class II protein.

26.   A method according to any one of claims 16 to 25,
wherein binding the mixture of synthetic heteropolymer
molecules further comprises providing heteropolymer
molecules having component amino acids selected from the
group consisting of: tyrosine (Y), phenylalanine (F),
valine (V), alanine (A), glutamic acid (E), and lysine
(K).

27.   A method according to any one of claims 24 to 26,
wherein synthesizing each peptide composition comprises
selecting amino acids from the group consisting of:
tyrosine (Y), phenylalanine (F), valine (V), alanine (A),
glutamic acid (E), and lysine (K).

28.   A method of treating an autoimmune disease
comprising the step of administering to a patient in need
thereof an effective amount of peptide, composition or
preparation according to any one of claims 1 to 27.

29.   Use of a peptide, composition or preparation
according to any one of claims 1 to 28, for the
preparation of a medicament for the treatment of
autoimmune disease.

30.   A peptide according to any one of claims 1 to 5,
substantially as herein described with reference to any

one of the examples.

31. A method for obtaining an MHC class II binding motif
amino acid sequence according to claim 9, substantially as
5   herein described with reference to any one of the
examples.

Dated this 3rd day of July 2003
10 THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE
By their Patent Attorneys
GRIFFITH HACK
Fellows Institute of Patent and
Trade Mark Attorneys of Australia
15