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(54) Title: MODIFIED PEPTIDES AND THEIR USE FOR THE TREATMENT OF AUTOIMMUNE DISEASES

(57) **Abrégé/Abstract:**

Disclosed herein are a modified peptide, compositions containing the same and their use in the treatment of autoimmune diseases. The modified peptide is provided by a chemical modification of at least one of the several amino acid residues comprising the peptide of sequence RIHMUYSKRSGKPRGYAFIEY (seq ID No : 1). The modification is carried out by phosphorylation, acetylation or methylation, or as a combination thereof.

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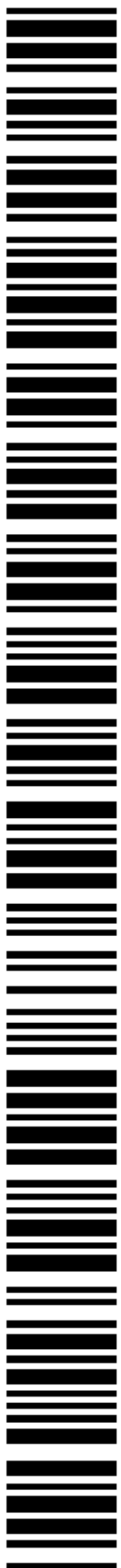
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(54) Title: MODIFIED PEPTIDES AND THEIR USE FOR THE TREATMENT OF AUTOIMMUNE DISEASES

(57) Abstract: Disclosed herein are a modified peptide, compositions containing the same and their use in the treatment of au-
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comprising the peptide of sequence RIHMUYSKRSGKPRGYAFIEY (seq ID No : 1). The modification is carried out by phospho-
rylation, acetylation or methylation, or as a combination thereof.

MODIFIED PEPTIDES AND THEIR USE FOR THE TREATMENT OF AUTOIMMUNE DISEASES
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FIELD OF THE INVENTION

[0002] The subject matter of the present invention is directed toward compositions and methods for the treatment of autoimmune diseases, specifically those diseases where it can be shown that the autoimmune process contributes to the pathogenesis of the disease.

BACKGROUND OF THE INVENTION

Autoimmune Diseases

[0003] These disorders may be looked upon as forming a spectrum. At one end, we have "organ-specific diseases" with organ-specific auto-antibodies.

Hashimoto's disease of the thyroid is an example. In this disorder, there is a specific lesion in the thyroid involving infiltration by mononuclear cells (lymphocytes, histiocytes and plasma cells), destruction of follicular cells and germinal center formation, accompanied by the production of circulating antibodies with absolute specificity for certain thyroid constituents. Towards the center of the spectrum are those disorders where the lesion tends to be localized to a single organ but the antibodies are non-organ-specific. A typical example would be primary biliary cirrhosis where the small bile ductule is the main target of inflammatory cell infiltration but the serum antibodies present-mainly mitochondrial-are not liver specific.

Systemic Lupus Erythematosus (SLE)

[0004] At the other end of the spectrum are the "non-organ-specific" or "systemic autoimmune diseases," broadly belonging to the class of rheumatological disorders, exemplified by systemic lupus erythematosus (SLE), where both lesions and auto-antibodies are not confined to any one organ. Pathological changes are widespread and are primarily lesions of connective tissue with fibrinoid necrosis. They are seen in the skin (the "lupus" butterfly rash on the face is characteristic), kidney glomeruli, joints, serous membranes and blood vessels. In addition, the formed elements of the blood are often affected. A bizarre collection of auto-antibodies are found, some of which react with the DNA and other nuclear constituents, of all cells in the body.

[0005] SLE predominantly affects women and is more common in blacks. Although survival rates have improved, over one half of patients with SLE have permanent damage in one or more organ systems. Arthritis and cutaneous manifestations are most common, but renal, hematologic and neurologic manifestations contribute largely to morbidity and mortality.

[0006] The pathophysiology of SLE is not completely understood. The production of abnormal antibodies by B cells remains the hallmark sign of SLE. Some of the auto-antibodies, such as anti-double-stranded DNA and anti-Smith, are very specific for SLE. Others, including anti-RNP, anti Rh_0 and anti-La, are also present in other autoimmune diseases. Whether the B cells themselves are intrinsically abnormal is a subject of current research. One of the underlying defects in SLE may center on apoptosis, or programmed cell death. In patients with SLE, cellular antigens exposed during apoptosis incite an immune response.

[0007] Examples of other major diseases considered to be associated with autoimmunity include: primary myxedema, pernicious anemia, Addison's disease, myasthenia gravis, juvenile diabetes, idiopathic thrombocytopenic purpura, ulcerative colitis, multiple sclerosis, rheumatoid arthritis, and scleroderma.

[0008] It has been proposed that a pre-existing network linked to epitopes of specific antigens exists and that malfunctioning of this network

[0009] A deregulation of apoptosis, for example in the execution phase, or in the clearance of apoptotic material by scavenger phagocytes (polymorphonuclear neutrophils macrophages), might predispose patients with appropriate genetic background to a break of tolerance toward cell-associated antigens and to the emergence of auto-immunity. An important question is to identify which antigen(s) and which epitope(s) may be relevant in the initiation of the autoimmune process. Proteins bearing these autoepitopes are present *in vivo* and it is possible to identify them as well as the relevant epitope(s).

[0010] Definitions:

- modified peptide: a chemical modification of one or several amino acid residues constituting the peptide such as, but not limited to, phosphorylation, acetylation, and methylation; the modification must allow the recognition of the modified peptide as an antigen by antigen-presenting cells (APC's), and, in this context, by auto-reactive CD4+T cells directed toward the naturally occurring, or possibly naturally modified, peptide (if the modification occurs naturally at the particular amino acid position as the peptide exists in the cell).
- Altered Peptide Ligands (APL): peptides recognized as antigens by APCs and, in the context of the present invention, by CD4+T cells, and able to induce a cascade of events different from the naturally occurring one (e.g., stimulation of other cells, production of different cytokines, difference in the signaling, etc.); this can lead to a significant diminution or even a complete cessation of the deleterious autoimmune response.
- Systemic lupus erythematosus (SLE): a syndrome of multifactorial etiology characterized by widespread inflammation in humans; in SLE, the body's natural defenses against infection are turned against the body through the production of antibodies against the body's own cells; these antibodies fight against the body's blood cells, organs and tissues, causing chronic diseases.

- epitope: that part of an antigen recognized by an antigen receptor.
- apoptosis: form of programmed cell death characterized by endo-nuclear digestion of DNA.

[0011] It has been proposed that a pre-existing network linked to epitopes of specific antigens exist and that malfunctioning of this network produces autoimmune disease states. Suggestions have been made that peptide analogs that will bind to the appropriate MHC molecule and block the response to autoantigens can be utilized to turn off an ongoing autoimmune response. This manner of proceeding has certain disadvantages including impairment of microbial defenses and the requirement for very high doses of the peptide.

SUMMARY OF THE INVENTION

[0012] The present invention, in one embodiment, provides a modified peptide in which at least one of the amino acid residues constituting the peptide has been chemically modified. Preferably, the modified peptides of the present invention are modified from the naturally-occurring peptides by one or more methods selected from the group consisting of phosphorylation, acetylation and methylation. Specifically contemplated by the present invention is a modified peptide, wherein the peptide is RIHMOVYSKRSGKPRGYAFIEY [SEQ ID NO: 1]. Preferably, the chemical modification of the peptide of SEQ ID NO: 1 is carried out by phosphorylation of S in position 7 and/or in position 10. Alternatively, the present invention provides the modified peptide of SEQ ID NO: 1 wherein the chemical modification is carried out by acetylation of K in position 8 and/or in position 12. In another alternative embodiment, the present invention provides a modified peptide of SEQ ID NO: 1, wherein the chemical modification is carried out by any combination of the phosphorylation of S in position 7 and/or 10, and the acetylation of K in position 8 and/or in position 12. In general, the present invention contemplates a modified peptide derived from a 70 kDa snRNP protein. In addition, the present invention provides the modified peptide of SEQ ID NO: 1 that has been phosphorylated in the 10 position.

[0013] In a second embodiment, the present invention contemplates a pharmaceutical composition in dosage unit form, comprising in combination a pharmaceutically acceptable carrier and an effective amount of at least one modified peptide of the invention effective for reducing or eliminating a deleterious autoimmune response. Preferably, the modified peptide is chemically modified RIHMOVYSGKPRGYAFIEY [SEQ ID NO: 1]. More preferably, the modified peptide of SEQ ID NO: 1 is chemically modified by any combination of the phosphorylation of S in position 7 and/or in position 10, and the acetylation of K in position 8 and/or in position 12. Alternatively, the composition of the present invention comprises a modified peptide of SEQ ID NO: 1 wherein the peptide is chemically modified by phosphorylation in the 10 position. In one aspect of this embodiment, the present invention provides a composition that is in the form of a lozenge, tablet, gelatin, capsule, drop, pill, or liposome. Alternatively, the composition is in the form of a solution.

[0014] In yet another alternative embodiment, the present invention encompasses a method for treating autoimmune diseases, wherein the method comprises the step of administering to a patient in need of such treatment a pharmaceutical composition comprising a modified peptide prepared according to the present invention in an amount sufficient to effect the desired treatment. In addition, the present invention contemplates a method of treating systemic lupus erythematosus that comprises administering to a patient in need of such treatment a pharmaceutical composition comprising a peptide modified according to the teachings of the present invention in an amount sufficient to effect said treatment.

[0015] The present invention also provides a modified peptide that is the product of the chemical modification by at least one of the methods selected from the group consisting of phosphorylation, acetylation, or methylation of RIHMOVYSGKPRGYAFIEY [SEQ ID NO: 1].

DETAILED DESCRIPTION OF THE INVENTION

[0016] In accordance with the practice of the present invention, it has been found that it is possible to create a family of modified peptides, wherein the therapeutically relevant epitopes of the peptides have been modified so as to transform the peptides into "altered peptide ligands" (APL's). These APL's are

capable of acting as decoys towards CD4+ T cells, thereby drastically reducing their immune effectiveness and, consequently, the organism's autoimmune response.

[0017] The APL's are created by a chemical modification of one or more of the several amino acid residues constituting the peptide including without limitation, and by way of example, phosphorylation, acetylation and methylation. The modification must allow recognition of the modified peptide as an antigen by antigen presenting cells and in this context by auto-reactive CD4+T cells directed against the naturally occurring, or possibly modified, peptides. The modification serves to convert the peptides into altered peptide ligands that are recognized as antigens by antigen-presenting cells and, in the practice of the instant invention, by CD4+ T cells, functioning to significantly reduce or entirely eliminate a deleterious autoimmune response.

[0018] Specifically, the present invention is directed toward a process that comprises modifying a therapeutically relevant epitope of a peptide recognized as an antigen by the CD4+ T cells that are responsible for inducing an autoimmune response. Furthermore, the present invention lies in the synthetic peptides defined as "modified" from their natural epitopes and able to demonstrate an effective activity against autoimmune diseases, and their preparation. In particular, the invention comprises modifications, including the phosphorylation of S in position 7 and/or 10; the acetylation of K in position 8 and/or 12; and any combination thereof, of the specific peptide RIHMOVYSKRSGKPRGYAFIEY (SEQ ID NO: 1), which peptide is a 21-amino acid segment of the 70 kDa snRNP protein, corresponding to residues 131 to 151.

[0019] The U1-small nuclear ribonucleoprotein particle (snRNP) belongs to the most complex autoantigens known to be recognized in systemic autoimmune diseases. This particle consists of an RNA backbone and eleven associated proteins that are immunogenic in patients suffering from systemic lupus erythematosus and mixed connective tissue disease (MCTD). Antibodies directed against the 70 kDa protein (also called U1 70 kDa or RNP-68) are specific for Sharp's syndrome. Up to 100% of all patients suffering from mixed connective tissue disease exhibit these autoantibodies. Apart from the 70 kDa protein and the Sm proteins, the U1-snRNP-complex also contains the proteins A and C. Patients suffering from SLE and MCTD exhibit antibodies directed

against the proteins A, C and 70 kDa. Autoantibodies against Sm proteins are of pathognomonic importance for diagnosing SLE. A patient with a positive anti-Sm finding is confronted with the diagnosis of SLE. But a negative finding does not exclude SLE. Anti-Sm is detected in 10% of Caucasian and 30% of Black and Chinese patients with SLE. The detection of autoantibodies against Sm belongs to the criteria for the diagnosis of SLE of the American College of Rheumatology (ACR) published in 1982.

[0020] Furthermore, the advantageous properties of the substances of the invention are accompanied by low toxicity.

[0021] These substances are particularly suited to the development of pharmaceutical compositions.

[0022] Other characteristics and advantages of the invention will become apparent from the example which follows relating to the preparation of the modified peptide and to the study of its activity against an autoimmune disease. Systemic lupus presents an easy clinical symptom to follow because of the large urinary protein excretion that accompanies the disorder that results from an associated renal condition, glomerulonephritis. Levels of protein secretion were followed to assess the effectiveness of the modified peptide in addressing the underlying autoimmune disorder.

Example 1

[0023] The specific peptide RIHMOVYSKRSGKPRGYAFIEY (SEQ ID NO: 1), which is a 21-amino acid sequence of the 70 kDa snRNP protein corresponding to residues 131 - 151, was synthesized in its phosphorylated form (in the 10 position - P10). This self-protein, which is normally present in each cell, is recognized by T-cells and antibodies from lupus patients and mice as an antigen toward which the immune system is reacting, generating a so-called autoimmune reaction. After multiple administrations (similar to a vaccine) in mice, the modified peptide induced a significant improvement in the survival rate (55% compared to 0%), whereas administration of the natural (non-phosphorylated) peptide, and the peptide phosphorylated in position 7, were unable to improve significantly the survival rate. Preliminary data of the acetylated peptide demonstrated an even stronger activity. Furthermore, the modified peptide P10

also increased significantly the production of IL-2 (interleukin-2) by CD4 + T cells and reduced the urine protein excretion in treated mice.

[0024] The pharmaceutical compositions of the invention contain an efficacious amount of a modified peptide in combination with an inert pharmaceutical vehicle.

[0025] In view of their ability to block the response to autoantigens, the compositions can be used in the treatment of those diseases associated with autoimmunity, including rheumatoid arthritis, Addison's disease, scleroderma, systemic lupus erythematosus, myasthenia gravis, juvenile diabetes, and the like.

[0026] The pharmaceutical compositions of the invention may be administered with different forms and by different routes, including nasal, rectal, oral and by injection. In the case of administration by the oral route, recourse may be had, in particular, to tablets, pills, lozenges, gelatin capsules, drops and even liposome. Other forms of administration comprise sterile or sterilizable solutions which can be injected by the intravenous, subcutaneous or intramuscular route.

[0027] In the case of the liposome form of administration, this method of presentation can be utilized to advantage by encapsulating the modified peptide in acid-resistant liposomes so that it can only enter the MHC class 1 route and stimulate CD8 + T cells. Antigens within acid-sensitive liposomes become associated with both class I and class II molecules and evidence high response efficiency. It should therefore be possible to use a single-shot liposome vaccine with multiple potentialities which incorporate several modified peptides, *i.e.*, antigens, different adjuvants and specialized targeted molecules for accomplishing the desired treatment.

[0028] For practical and economic reasons, whatever route is selected, the minimum number of doses and the least amount of modified peptide should be involved.

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<213> Homo sapiens

<400> 1

Arg Ile His Met Val Tyr Ser Lys Arg Ser Gly Lys Pro Arg Gly Tyr
1 5 10 15

Ala Phe Ile Glu Tyr
20

Claims

1. A modified peptide , or a salt thereof, consisting of SEQ ID NO :1
RIHMOVYSGKPRGYAFIEY [SEQ ID NO: 1],
wherein S in position 10 is modified by phosphorylation.
2. A pharmaceutical composition in unit dosage form, comprising in combination a
pharmaceutical acceptable carrier and at least one modified peptide, or a salt thereof,
according to claim 1.
3. The composition according to claim 2 which is in the form of a lozenge, tablet,
gelatin, capsule, drop, pill, or liposome, or a solution.
4. Use of a modified peptide, or a salt thereof, according to claim 1, for the preparation
of a medicament for treating an autoimmune disease.
5. Use of a modified peptide according to claim 4, wherein said autoimmune disease is
systemic lupus erythematosus