Title: IMMUNOGENIC T CELL TARGETS IN AUTOIMMUNE HEPATITIS AND METHODS OF USE

Abstract: The present disclosure takes advantage of the discovery of several T cell epitopes involved in autoimmune hepatitis (AIH). This discovery makes possible new methods of diagnosing and monitoring the progression or response to therapy of a subject with AIH, as well as kits for diagnosing and monitoring the disease, and methods of treating AIH.
IMMUNOGENIC T CELL TARGETS IN AUTOIMMUNE HEPATITIS AND
METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application
No. 60/659,513, filed March 7, 2005, which application is incorporated herein in its
entirety.

FIELD OF THE DISCLOSURE

The present disclosure relates to T cell epitopes involved in autoimmune
hepatitis (AIH). The disclosure further relates to new methods of diagnosing and
monitoring the progression or response to therapy of a subject with AIH, as well as kits
for diagnosing and monitoring the disease, and methods of treating AIH.

BACKGROUND

Elucidating the pathogenesis of autoimmune diseases has challenged
immunologists for decades. Three to five percent of the population in Western nations
suffers from autoimmune diseases (Jacobson et al., Clin. Immunol. Immunopathol.,
84:223-243, 1997). Whereas this number may be considered relatively small, it
represents an immense medical and socioeconomic burden.

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease, which has
a prevalence of approximately 1/10,000 in the United States and Europe and accounts
for 2-6% of all liver transplantations in these countries (Milkiewicz et al.,
Transplantation, 68:253-6, 1999). It is typically diagnosed by elevated alanine amino
transferase levels and an intrahepatic lymphocytic mononuclear cell infiltrate in the
liver, by hypergammaglobulinemia and autoantibodies, and by the absence of liver
disease of viral, toxic or metabolic etiology (Milkiewicz et al., Transplantation,

Autoantibodies target cell nuclei (antinuclear antibodies, ANA), smooth muscles
(SMA), liver-kidney microsomes (LKM-1) and/or soluble liver antigen/liver-pancreas antigen (SLA/LP). SLA/LP-specific antibodies, detectable in about 20% of AIH cases (Herkel et al., Hepatology, 35:403-8, 2002; Manns et al., Lancet, 1:292-4, 1987), are unique in that they are the only autoantibodies that are specific for AIH (Herkel et al., Hepatology, 35:403-8, 2002). Although autoantibodies are well accepted as serologic markers for diagnosis and classification of AIH, it is still unclear which role they play in the pathogenesis of the disease (Lapierre et al., Hepatology, 39:1066-74, 2004; Yamauchi et al., Hepatology, 42:149-55, 2005).

Liver-infiltrating lymphocytes, especially plasma cells and CD4+ and CD8+ T cells that are located at the interface between liver parenchyma and connective tissue, are the histological hallmark of AIH. It has been suggested that intrahepatic T cells contribute to the pathogenesis of AIH by recognizing self-antigens in the context of HLA-DRB1*0301 and HLA-DRB1*0401 (McFarlane, Semin. Liver. Dis., 22:317-24, 2002). This idea is supported by the clonal restriction of the intrahepatic T cell population and by the observation that HLA-DRB1*0301 and HLA-DRB1*0401 predispose to AIH and influence disease severity (Donaldson et al., Hepatology, 13:701-6, 1991; Czaja et al., Hepatology, 25:317-23, 1997). HLA-DRB1*0301, the principal AIH susceptibility allele among white Europeans and Americans (Donaldson et al., Hepatology, 13:701-6, 1991; Czaja et al., Hepatology, 25:317-23, 1997), and HLA-DRB1*0401 might even present the same autoantigen to CD4+ T cells because both alleles share an amino acid motif in their antigen binding groove ("shared binding hypothesis") (Czaja et al., Immunol. Rev., 174:250-9, 2000).

The identification of antigenic peptides that are presented by HLA-DRB1*0301 and HLA-DRB1*0401 in AIH has presented a difficult challenge. As in other autoimmune diseases (Reijonen et al., Diabetes, 53:1987-94, 2004), the frequency of autoreactive CD4+ T cells in the peripheral blood is too low to screen a large number of candidate peptides for T cell recognition. Although the relative frequency of autoreactive CD4+ T cells is presumably higher at the site of inflammation, only a small piece of tissue can be obtained by liver biopsy. Therefore, it is necessary to expand
and/or clone liver-biopsy-derived T cells \textit{in vitro} before they can be studied in functional assays (Lohr \textit{et al.}, \textit{Hepatology}, 24:1416-21, 1996). This approach renders an \textit{ex vivo} assessment of the number and function of autoantigen-specific T cells impossible. Furthermore, assays that solely rely on the function of autoantigen-specific T cells are often compromised due to immunosuppressive therapy of patients with chronic AIH.

Agents are needed that permit the \textit{ex vivo} identification and quantification of AIH-specific T cells irrespective of their function. Such agents can be used, for example, to monitor AIH-specific T cell responses in the peripheral blood of patients with AIH, for instance, during immunosuppressive therapy, and to treat AIH.

\textbf{SUMMARY OF THE DISCLOSURE}

This disclosure involves the discovery of isolated SLA/LP epitopes that serve as T cell targets in autoimmune hepatitis (AIH), altered peptide ligands and antagonists derived from the isolated SLA/LP T cell epitopes, and isolated multimeric MHC complexes including such SLA/LP T cell epitopes or altered peptide ligands.

The disclosed compositions enable a variety of kits and diagnostic and treatment methods; for example, methods of determining the quantity of T cells in a sample that are reactive with an isolated SLA/LP T cell epitopes or a multimeric MHC-SLA/LP T cell epitope complex. Exemplary methods of treating autoimmune hepatitis involve administering to a subject or exposing cells obtained or isolated from a subject to a therapeutically effective amount of altered peptide ligands or antagonists derived from disclosed SLA/LP T cell epitopes. In applicable methods, treated cells are then reintroduced into the subject. Other treatment methods involve administering to a subject or exposing cells obtained or isolated from the subject to a therapeutically effective amount of a multimeric MHC-SLA/LP T cell epitope complex, which complex includes a toxin moiety or a separation moiety that ablates or depletes T cells reactive with the SLA/LP T cell epitope. In applicable methods, cells treated in this manner are reintroduced into the subject.
The foregoing and other features and advantages of the disclosure will become more apparent from the following detailed description of a several embodiments which proceeds with reference to the accompanying figures. In the following Brief Description of the Figures and throughout the disclosure, SLA/LP peptides (e.g., SLA/LP T cell epitopes) may be referred to as “SLA_{aa1-aa2}” where aa1 and aa2 are the positions in the SLA/LP protein (SEQ ID NO: 6) of the first and last amino acid residue of the SLA/LP peptide.

**BRIEF DESCRIPTION OF THE FIGURES**

**FIG. 1** is a schematic representation of exemplary methods for identifying T cell-specific SLA/LP epitopes and for using such epitopes to identify SLA/LP-specific T cells.

**FIG. 2** is a set of three graphs showing the humoral immune response of SLA/LP-immunized HLA-DRB1*0301-transgenic mice. HLA-DRB1*0301 transgenic mice (n=16) were immunized with SLA/LP in adjuvant on day 0 and day 21 and SLA/LP-specific antibody responses were quantified at the indicated time points (FIG. 2A) and studied for isotype distribution on day 42 (FIG. 2B). The dashed horizontal line in FIG. 2A indicates the cut-off of positivity. FIG. 2C shows the reactivity of the indicated SLA/LP carboxy-terminal peptides with antibodies of immunized HLA-DRB1*0301 transgenic mice (filled bars) and C57BL/6 mice (open bars). Mean and standard deviation of the results of 3 mice are shown in FIGs. 2B and 2C. A p-value of <0.05 (Student t test) was considered significant.

**FIG. 3A** is a graph showing HLA-DR expression of HLA-DRB1*0301 transgenic mice prior to immunization. The histogram is gated on CD19+ B cells. **FIG. 3B** is a graph showing SLA/LP-specific, proliferative response of draining lymph node cells. The mean stimulation index and standard deviation of the responses of five mice are shown.


**FIG. 4** includes four panels (A-D) further illustrating the cellular immune response of SLA/LP-immunized HLA-DRB1*0301-transgenic mice. FIGs. 4A and 4B show SLA/LP dose-dependent proliferation (A) and IL-2 ELISpot (B) of spleen cells from a representative SLA/LP-immunized mouse (mean of triplicate values). FIG. 4C is a bar graph mapping the responses of T cells isolated from SLA/LP-immunized mice in response to stimulation by the indicated SLA/LP peptides. The mean number of IL-2 spot forming spleen cells (SFCs) of a group of 11 mice is shown. Responses higher than the mean plus 3 SD of the medium control are indicated by black bars. Immunogenic regions for which T cell hybrids could be established from either SLA/LP protein- or peptide-immunized mice are indicated by black horizontal bars in FIG. 4D, open horizontal bars represent regions for which T cell hybrids were not established.

**FIG. 5** shows the human SLA/LP amino acid sequence (SEQ ID NO: 6). Exemplary immunodominant epitopes are shown by solid underline. The amino acid sequence recognized by human SLA/LP-specific antibodies is shaded.

**FIG. 6** includes three panels showing the mapping of T cell epitopes encompassed by amino acid residues 369-388 of SLA/LP. CD4⁺ T cell hybrids specific for the 20mer SLA369-388 were stimulated (FIG. 6A) with the indicated truncated peptides (with reference to amino acid residues of SEQ ID NO: 6) in a dose-dependent manner (FIG. 6B). Exemplary epitope SLA373-386 had the highest relative activity. FIG. 6C shows that the T cell response to the SLA373-386 peptide was specifically blocked by antibodies specific for HLA-DR.

**FIG. 7** includes three panels showing the mapping of a T cell epitopes encompassed by amino acid residues 184-204 of SLA/LP. CD4⁺ T cell hybrids specific for the 20mer SLA185-204 were stimulated (FIG. 7A) with the indicated peptides (with reference to amino acid residues of SEQ ID NO: 6) in a dose-dependent manner (FIG. 7B). Exemplary epitope SLA186-197 had the highest relative activity. FIG. 7C shows that the T cell response to the SLA186-197 peptide was specifically blocked by antibodies specific for HLA-DR.
FIG. 8 shows a series of flow cytometry profiles. DRB1*0301/SLA\textsubscript{373-386} tetramer-positive (A) and DRB1*0301/SLA\textsubscript{184-198} tetramer-positive CD4\textsuperscript{+} T cells (B) were detectable in the spleens of representative SLA/LP-immunized DRB1*0301-transgenic mice, but not in the spleens of non-immunized DRB1*0301-transgenic mice. CD62L- and CD11a expression were compared on tetramer-positive and –negative CD4\textsuperscript{+} T cells. Numbers in the quadrants indicate the percentage of positive cells.

FIG. 9 shows the detection and characterization of SLA\textsubscript{373-386}-specific CD4\textsuperscript{+} T cells in humans using a DRB1*0301/SLA\textsubscript{373-386} tetramer. PBMC from a DRB1*0301-positive patient with anti-SLA/LP-positive AIH (A) and a DRB1*0301-positive control patient, who had recovered from hepatitis B virus infection but did not suffer from AIH (B) were labelled with CFSE and stimulated with the indicated peptides. The frequency of CD3\textsuperscript{+}CD8\textsuperscript{+} DRB1*0301/SLA\textsubscript{373-386} tetramer-positive T cells was assessed on day 10 of culture. Tetramer-positive and -negative cells were compared as regards to proliferation. Numbers indicate the percentage of cells in the respective quadrant.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

The nucleic acid and protein sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and triple letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO: 1 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (CFWRRFIHGIGR; also residues 7-18 of SEQ ID NO: 6).

SEQ ID NO: 2 shows an amino acid sequence containing a T cell-reactive SLA/LP epitope (IIWPRIDQKSCFK; also residues 86-98 of SEQ ID NO: 6).

SEQ ID NO: 3 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (IQQGARVGRID; also residues 187-197 of SEQ ID NO: 6).
SEQ ID NO: 4 shows an amino acid sequence containing a T cell-reactive SLA/LP epitope (EEMALKLDNVLLDTRYQD; also residues 403-419 of SEQ ID NO: 6).

SEQ ID NO: 5 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (NRLDRCLKAVRKE; also residues 373-386 of SEQ ID NO: 6).

SEQ ID NO: 6 shows a human SLA/LP sequence (GenBank Accession No. AAD33963, version 2 (a.k.a., GI:7652498).

SEQ ID NO: 7 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (LIQQGARVGRID; also residues 186-197 of SEQ ID NO: 6).

SEQ ID NO: 8 shows a DRB*-restricted hepatitis C virus peptide (VLVLNPSVA).

SEQ ID NO: 9 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (IQQQARVGR; also residues 187-196 of SEQ ID NO: 6).

SEQ ID NO: 10 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (RLDRCLKAVRKE; also residues 374-385 of SEQ ID NO: 6)

SEQ ID NO: 11 shows the amino acid sequence of an exemplary T cell-reactive SLA/LP epitope (MHLLIQGARVGRID; also residues 184-197 of SEQ ID NO: 6)

SEQ ID NO: 12 shows the amino acid sequence of an expected T cell-reactive SLA/LP epitope (CFWRRGKCPENG)

SEQ ID NO: 13 shows the amino acid sequence of an expected T cell-reactive SLA/LP epitope (RRHYRFIHYGGR)

SEQ ID NO: 14 shows an amino acid sequence that is inserted between residues 5 and 6 of the Jurkat cell SLA/LP protein sequence (SEQ ID NO: 6) to produce the human liver SLA/LP protein sequence.

DETAILED DESCRIPTION

I. Introduction

The present disclosure takes advantage of the discovery of several T cell epitopes involved in autoimmune hepatitis (AIH). This discovery makes possible new
methods of diagnosing and/or monitoring the progression of AIH and/or the response to therapy of a subject with AIH, as well as kits for diagnosing and monitoring the disease, and methods of treating AIH.

Disclosed are several isolated epitopes that serve as T cell targets in autoimmune hepatitis (AIH). Exemplary AIH-specific T cell epitopes include an amino acid sequence as set forth in SEQ ID NO: 1-5, 7, or 9-11, sequences having at least 90% or 95% sequence identity with the sequences shown in SEQ ID NO: 1-5, 7, or 9-11, and conservative variants of the sequences shown in SEQ ID NO: 1-5, 7, or 9-11. The immunogenic region peptides (epitopes) are each recognizable by T cells. In some embodiments, the peptides are 50 or fewer residues in length, for instance 30, 25 or 20 or fewer residues in length. In particular examples of the disclosure, the peptide is the sequence shown in SEQ ID NO: 1-5, 7, or 9-11.

Other embodiments of the disclosure are altered peptide ligands (APLs) derived from the isolated immunogenic region peptide described above. The altered peptide ligands include at least one amino acid substitution in a disclosed immunogenic region peptide, yet the altered peptide ligands still retain the ability to stimulate T cells. In some embodiments, the APL is 30 or fewer residues in length, for example 25 or 20 or fewer residues in length. In some embodiments, the amino acid substitutions are conservative substitutions, whereas in other embodiments, the amino acid substitutions are non-conservative substitutions.

Also disclosed are antagonists derived from the isolated immunogenic region peptide described above. The antagonists include an immunogenic region recognizable by T cells, and they antagonize an activity of the isolated immunogenic region peptide. In some embodiments the antagonist is a peptide antagonist, whereas in other embodiments, the antagonist is a synthetic, non-peptide antagonist.

Also disclosed is a method of diagnosing or monitoring an autoimmune disease. The method includes obtaining a sample from a subject and determining the quantity (e.g., number) of T cells in the sample that are reactive with a disclosed immunogenic
region peptide (epitope). In some embodiments, the sample is blood, lymphatic tissue, spleen tissue, or liver tissue.

Some embodiments of the method further include determining the function of T cells in the sample that are reactive with the isolated immunogenic region peptide described above. In some examples, determining the function of T cells in the sample includes detecting or measuring secreted or intracellular cytokines, detecting or measuring proliferation, detecting or measuring cytotoxicity, or detecting or measuring functional interaction with B cells, CD8 cells or dendritic cells.

Other embodiments of the method further include comparing a quantity of T cells reactive with the isolated immunogenic region peptides described above with a baseline. In particular examples, the baseline is a quantity of T cells reactive with the isolated immunogenic region peptides described above measured in a sample taken from the same subject at an earlier time. In other examples, the baseline is the average quantity (e.g., number) of T cells reactive with the isolated immunogenic region peptides described above measured in samples taken from a population of subjects with autoimmune hepatitis. In yet other examples, the baseline is the average quantity (e.g., number) of T cells in samples taken from a population of subjects without autoimmune hepatitis that are reactive with the isolated immunogenic region peptides described above. In particular examples of the method, determining the quantity of T cells in the sample that are reactive with the isolated immunogenic region peptides described above involves using a cytokine ELISpot assay, whereas in other particular examples it is carried out with a multimeric MHC/peptide antigen binding complex in which the peptide antigen includes the isolated immunogenic region peptide described above.

Another embodiment of the disclosure is a kit for diagnosing or monitoring autoimmune hepatitis in a subject that includes one of the AIH epitopes described above, a container, and instructions for using the kit.

Also disclosed herein are methods of treating autoimmune hepatitis in a subject. One method includes administering to the subject a therapeutically effective
amount of the altered peptide ligand described above, thereby treating the autoimmune hepatitis. Administering the altered peptide ligand changes the subject's predominant immune response to the epitope (for example, from a Th1 response to a Th2 response or vice versa), and thereby treats the disease. In some embodiments of the method, administering the isolated immunogenic region includes parenteral administration, for example sub-cutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery. In other embodiments, the method includes repeated administration, for instance daily, weekly, or monthly administration.

Yet other methods of treatment involve administering to the subject a therapeutically effective amount of the antagonist described above, thereby treating the autoimmune hepatitis. Administering the antagonist blocks the function of the T cells that target the immunogenic region peptides and thereby treats the disease. In some embodiments, administering the antagonist includes parenteral administration, for example, sub-cutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery. In certain examples, the antagonist is administered repeatedly, for instance daily, weekly, or monthly.

Yet still other methods include administering to the subject a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex, wherein the peptide antigen includes the isolated immunogenic region peptide described above, and wherein the multimeric MHC/peptide antigen complex is conjugated to a toxin moiety. Exposure to the toxin moiety specifically ablates the targeted activated T cells, thereby treating the autoimmune hepatitis in the subject. In some examples, administering the multimeric MHC/peptide antigen complex includes parenteral administration, for example, sub-cutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery. In other examples, the multimeric MHC/peptide antigen complex is administered repeatedly, for example daily, weekly, or monthly. In particular examples, the toxin moiety is ricin,
abrin, diphtheria toxin, a maytansinoid, or cisplatin, and in even more particular examples, the multimeric MHC/peptide antigen complex is a dimeric, trimeric, tetrameric, or pentameric complex.

Other methods of treatment include isolating immune cells from the subject, ablating T cells reactive with the isolated immunogenic region peptide described above with a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex, and re-introducing the immune cells to the subject. In these embodiments, the peptide antigen includes the isolated immunogenic region peptide described above, and the multimeric MHC/peptide antigen complex is conjugated to a toxin moiety.

Exposure to the toxin moiety selectively ablates the reactive T cells and thereby treats the autoimmune hepatitis in the subject. In particular examples, the toxin moiety is ricin, abrin, diphtheria toxin, a maytansinoid, or cisplatin, and in even more particular examples, the multimeric MHC/peptide antigen complex is a dimeric, trimeric, tetrameric, or pentameric complex.

Still another method of treating autoimmune hepatitis includes isolating immune cells from the subject, depleting T cells reactive with the isolated immunogenic region peptide described above with a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex, and re-introducing the immune cells to the subject. The peptide antigen includes a disclosed T cell-specific SLA/LP epitope, and the multimeric MHC/peptide antigen complex is conjugated to a separation moiety. The separation moiety permits the selective depletion of T cells reactive with the peptide epitope, thus reintroduction of the depleted immune cells treats the autoimmune hepatitis. In particular examples of the method, the separation moiety is phycoerythrin.

II. Abbreviations

AA: amino acid
AEC: 3-amino-9-ethylcarbazole
AIH: autoimmune hepatitis
ANA: antinuclear antibody
BSA: bovine serum albumen
CPRG: chlorphenol red reagent
DMSO: dimethyl sulfoxide
EIA: ELISA Immunoassay
ELISpot: enzyme-linked immunospot
IL-2: Interleukin-2
LKM: liver-kidney microsomes
MPL: monophosphoryl lipid
NFAT: nuclear factor of activated cells
PBS: phosphate buffered saline
PMA: phorbol myristic acetate
SDS-PAGE: sodium dodecylsulfate polyacrylamide gel electrophoresis
SFC: spot forming cells
SLA/LP: soluble liver antigen/liver pancreas antigen
SMA: smooth muscle antigen
TCR: T cell receptor
TMB: tetramethylbenzidine

III. Terms

Unless otherwise noted, technical terms are used according to conventional usage.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

**Altered peptide ligands (APL):** Variant peptide epitopes in which at least one but no more than about 30% of the amino acid residues of the epitope are substituted with different amino acid residues, yet the APLs still bind to the same MHC molecules as the wild-type epitopes and activate T cells involved in a disease process (such as, AIH). For example, in some embodiments from about one to about six amino acid residues are different from the corresponding residues of the wild-type epitope (such as from about one to about five, from about one to about four, from about one to about three, one or two, or only one residue(s) differ from a disclosed SLA/LP T cell epitope). Methods of therapy and prophylaxis involving APLs are described, e.g., in U.S. Patent No 6,562,943. In some embodiments, APLs based on the specific T cell epitopes described herein are used to treat and/or prevent autoimmune hepatitis.

APLs have the ability to elicit different patterns of cytokine production in CD4 T cells than do their parent wild-type peptides. Thus, for example, while a wild-type peptide may induce production of Th1 cytokines, an APL derived from it may elicit Th2 cytokines. Alternatively, the wild-type peptide may stimulate the production of Th2 cytokines and a corresponding APL elicits production of Th1 cytokines.

**Anergy:** A state of T cell unresponsiveness. Anergy can be induced by, among other things, stimulation of the T cell’s antigen receptor in the absence of costimulatory signals.

**Animal:** Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term “subject” includes both human and veterinary subjects.

**Antagonist:** An agent that opposes the physiological effects of another. At the receptor level, an antagonist is an agent that opposes the receptor-associated responses normally induced by another bioactive agent. As used herein, a T cell receptor antagonist is a peptide or natural or artificial non-peptide ligand that competitively inhibits the specific binding of an protein or peptide epitope (such as a disclosed
SLA/LP T cell epitope or APL) to a T cell receptor and thereby blocks (antagonizes) T cell receptor function.

**Antigen:** A compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal. The definition includes compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. An “antigen” includes one or more antigenic epitopes, including, for example, a single epitope or a combination of epitopes. Epitopes constituting a combination of epitopes may be derived from a single compound, composition, or substance or from different compounds, compositions, or substances. The individual epitopes of a combination epitope may be directly linked one to the other (using, for example, recombinant techniques commonly known in the art) or individual epitopes of a combination epitope may be linked together via linkers (such as, relatively short, non-antigenic peptide sequence) that separate the individual epitopes. Antigens can also include fragments of known antigens that retain the ability to stimulate the production of antibodies or a T cell response in an animal. For example, an immunogenic composition of the type described herein may include a peptide of at least about 5, 10, 15 or 20 amino acid residues. Smaller immunogens may require the presence of a “carrier” polypeptide, for example as a fusion protein, aggregate, conjugate or mixture linked (chemically or otherwise) to the immunogen.

**Antigen presenting cell (APC):** A class of cells capable of presenting one or more antigens in the form of an antigen-MHC complex recognizable by specific effector cells of the immune system, and thereby inducing an effective cellular immune response against the antigen or antigens being presented. An APC cell carries on its surface antigen bound to MHC Class I or Class II molecules, and presents the antigen in this “context” to T cells. Examples of antigen presenting cells include, but are not limited to macrophages, dendritic cells (DC), follicular dendritic cells (FDC), and Langerhans cells. Macrophages are large white blood cells that ingest antigens and other foreign substances. Each macrophage contains packets of chemicals and enzymes that digest
the ingested antigen or microbe. Dendritic cells are the principle APC involved in primary immune responses. Their major function is to obtain antigen in tissues, migrate to lymphoid organs and activate T cells. Langerhans cells are dendritic cells specific to the skin.

**Autoimmune disorder:** A disorder in which the immune system produces an immune response (for instance, a B cell and/or a T cell response) against an endogenous antigen, with consequent injury to tissues. Specific, non-limiting examples of autoimmune diseases include type I diabetes, multiple sclerosis, rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosus, autoimmune premature ovarian failure, Graves’ thyroiditis, Hashimoto’s thyroiditis, primary hypothyroidism, celiac disease, primary biliary cirrhosis, autoimmune hepatitis, Addison’s disease, vitiligo, systemic sclerosis, and anti-glomerular basement membrane disease.

Autoimmune disorders can be classified into two broad, but overlapping, groups: organ-specific and non-organ-specific (or systemic) autoimmune disorders. In the first mentioned, local injury, inflammation, or dysfunction are produced by autoantibody- or cell-mediated reactions against a specific target antigen located in a specialized cell, tissue, or organ. Clinical examples include graft versus host disease, autoimmune hemolytic anemia (erythrocyte autoantibodies), Hashimoto’s thyroiditis (thyroid autoantibodies and autoreactive T cells), myasthenia gravis (acetylcholine receptor autoantibodies), Grave’s disease characterized by diffuse goiter and hyperthyroidism (thyrotropin receptor autoantibodies), Goodpasture’s syndrome comprising anti-glomerular basement membrane (GBM) nephritis and pulmonary intraalveolar hemorrhage (anti-GBM autoantibodies), and type I (insulin-dependent) diabetes (pancreatic beta-cell autoreactive T cells and autoantibodies).

In a systemic autoimmune disease, by contrast, tissue injury and inflammation occur in multiple sites in organs without relation to their antigenic makeup and are usually initiated by the vascular leakage and tissue deposition of circulating autologous immune complexes. These immune complexes are formed by autoantibody responses to ubiquitous soluble cellular antigens of nuclear, or less commonly cytoplasmic,
origin. Systemic lupus erythematosus, which is characterized by the production of multiple autoantibodies, particularly antinuclear and anti-DNA antibodies, is the classical example of an immune complex-mediated systemic autoimmune disease.

**cDNA (complementary DNA):** A DNA sequence lacking internal, non-coding segments (introns) and transcriptional regulatory sequences. cDNA can also contain untranslated regions (UTRs) that are responsible for translational control in the corresponding RNA molecule. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

**Chemokine:** A chemokine is a small chemoattractant protein that stimulates the migration and activation of cells, especially phagocytic cells and lymphocytes. Chemokines have a central role in inflammatory responses.

**Cross-linker:** An agent or process that forms chemical bonds between previously separate molecules. In some embodiments, cross-linking is accomplished by heat, vulcanization, irradiation or the addition of a suitable chemical agent, for example glutaradehyde.

**Cytokine:** A cytokine is a protein made by cells that affect the behavior of other cells. Cytokines act on specific cytokine receptors on the cells that they affect.

**Dendritic cells:** A diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues. Dendritic cells constitute the most potent APCs in an organism. A subset of dendritic cells are derived from bone marrow progenitor cells, circulate in small numbers in the peripheral blood and appear either as immature Langerhans' cells or terminally differentiated mature cells. Dendritic cells do not have the CD14 antigen marker associated with monocytes.

Dendritic cells recognize and act against invading antigens of the lymphoid and hematopoietic systems and skin, and function as the principle APC involved in primary immune responses. Their major function is to obtain antigen in tissues, migrate to lymphoid organs and activate T cells, thereby stimulating cellular immunity. Dendritic cells are also known as interdigitating, reticular, and veiled cells.
DNA (deoxyribonucleic acid): A long chain polymer that comprises the genetic material of most living organisms (some viruses have genes comprising ribonucleic acid (RNA)). The repeating units in DNA polymers are four different nucleotides, each of which comprises one of the four bases, adenine, guanine, cytosine and thymine bound to a deoxyribose sugar to which a phosphate group is attached. Triplets of nucleotides (referred to as codons) code for each amino acid in a polypeptide. The term codon is also used for the corresponding (and complementary) sequences of three nucleotides in the mRNA into which the DNA sequence is transcribed.

Unless otherwise specified, any reference to a DNA or RNA molecule is intended to include the reverse complement of that molecule. Except where single-strandedness is required by the text herein, DNA or RNA molecules, though written to depict only a single strand, encompass both strands of the molecule. Thus, a reference to the nucleic acid molecule that encodes a specific protein, or a fragment thereof, encompasses both the sense strand and its reverse complement. Thus, for instance, it is appropriate to generate probes or primers from the reverse complement sequence of the disclosed nucleic acid molecules.

Encode: A polynucleotide is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

Epitope: The portion(s) of an antigen molecule which contact the antigen binding site of an antibody or T cell receptor (also referred to as an antigenic determinant). An epitope can be a sequential or non-sequential (conformational) portion of an antigen molecule. An epitope also may be formed through intramolecular or intermolecular combinatorial (for example, non-linear, tertiary, or quaternary) associations. An antibody or T cell binds a particular antigenic epitope. T cells recognize an epitope that is presented in association with specific MHC molecules. An
epitope can include from about 5 to about 30 (consecutive or non-consecutive) amino acids (such as, from about 5 to about 20 amino acids, from about 5 to about 15 amino acids, or from about 5 to about 12 amino acids).

**Expression:** A process by which mRNA is translated into peptides, polypeptides or proteins. Expression can also include transcription of polynucleotides from DNA into mRNA, or translation directly from RNA, for example an RNA replicon.

**Hybridization:** Oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, a nucleic acid molecule consists of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as “base pairing.” More specifically, A will hydrogen bond to T or U, and G will bond to C. “Complementary” refers to the base pairing that occurs between to distinct nucleic acid sequences or two distinct regions of the same nucleic acid sequence.

**Immune response:** A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an “antigen-specific response”). An immune response can be a humoral (antibody or B cell response) or cellular (cell mediated or T cell response). In one embodiment, an immune response is a T cell response, such as a CD4+ T cell response or a CD8+ T cell response.

**Immunosuppressive agent:** A molecule, such as a chemical compound, small molecule, steroid, nucleic acid molecule, or other biological agent, that can decrease an immune response such as an inflammatory reaction. Immunosuppressive agents include, but are not limited to an agent of use in treating or preventing an autoimmune disorder, for example arthritis, autoimmune hepatitis, multiple sclerosis, or type 1 diabetes. Specific, non-limiting examples of immunosuppressive agents are
non-steroidal anti-inflammatory agents, FK506, and anti-CD4. In additional examples, the agent is a biological response modifier, such as Kineret® (anakinra), Enbrel® (etanercept), or Remicade® (infliximab), a disease-modifying anti-rheumatic drug (DMARD), such as Arava® (lefllunomide), a non-steroidal anti-inflammatory drug (NSAID), specifically a Cyclo-Oxygenase-2 (COX-2) inhibitor, such as Celebrex® (celecoxib) and Vioxx® (rofecoxib), or another product, such as Hyalgan® (hyaluronan) and Synvisc® (hylan G-F20). Also of use are cyclophosphamide (Cytoxan®), mitoxantrone (Novantrone®), azathioprine (Imuran®), cladribine (Leustatin®), cyclosporine-A, and methotrexate, as well as the immunomodulators beta interferon 1b (Betaseron®), beta interferon 1a- intramuscular (Avonex®), beta interferon 1a- subcutaneous (Rebif®), and glatiramer acetate (Copaxone®).

**Isolated:** An “isolated” biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, for example, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins which have been “isolated” thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides, and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

**Leukocyte:** Cells in the blood, also termed “white cells,” that are involved in defending the body against infective organisms and foreign substances. Leukocytes are produced in the bone marrow. There are five main types of white blood cells, subdivided between two main groups: polymorphonuclear leukocytes (neutrophils, eosinophils, basophils) and mononuclear leukocytes (monocytes and lymphocytes). When an infection is present, the production of leukocytes increases.

**Major Histocompatibility Complex (MHC):** A group of genes that control the expression of cell surface protein markers (also known as human leukocyte antigen or HLA markers) that determine T cell receptor shape and allow immune cells to recognize components of the body (for example, to distinguish “self” from “non-self”). MHC
molecules are necessary for antigen presentation and for recognition of antigens by
immune system cells. Each individual has one of a variety of genetically-determined
MHC/HLA patterns. There are two classes of MHC molecules, MHC-I and MHC-II.
The MHC genes were originally defined as the genetic locus coding for those
cell surface antigens presenting the major barrier to transplantation between individuals
of the same species. They include a cluster of genes on human chromosome 6 or mouse
chromosome 17 that encodes the MHC molecules. MHC class I proteins present
peptides generated in cytosol to CD8 T cells, and MHC class II proteins present peptides
degraded in cellular vesicles to CD4 T cells. The MHC also encode proteins involved
in antigen processing and host defense. The MHC is the most polymorphic gene cluster
in the human genome, having large numbers of alleles at several different loci. Because
this polymorphism is usually detected using antibodies or specific T cells, the MHC
proteins are often called major histocompatibility antigens.

**Mammal:** This term includes both human and non-human mammals.

Similarly, the term subject includes both human and veterinary subjects.

**Nucleotide:** This term includes, but is not limited to, a monomer that includes a
base linked to a sugar, such as a pyrimidine, purine, or synthetic analogs thereof, or a
base linked to an amino acid, as in a peptide nucleic acid (PNA). A nucleotide is one
monomer in a polynucleotide. A nucleotide sequence refers to the sequence of bases in
a polynucleotide.

**Nucleic acid:** A deoxyribonucleotide or ribonucleotide polymer in either single
or double stranded form, and unless otherwise limited, encompasses known analogues
of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally
occurring nucleotides.

**Oligonucleotide:** A plurality of nucleotides joined by native phosphodiester
bonds, between about 6 and about 300 nucleotides in length. An oligonucleotide analog
refers to moieties that function similarly to oligonucleotides but have non-naturally
occurring portions. For example, oligonucleotide analogs can contain non-naturally
occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a
phosphorothioate oligodeoxynucleotide. Functional analogs of naturally occurring polynucleotides can bind to RNA or DNA, and include peptide nucleic acid (PNA) molecules.

Particular oligonucleotides and oligonucleotide analogs can include linear sequences up to about 200 nucleotides in length, for example a sequence (such as DNA or RNA) that is at least 6 bases, for example at least 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100 or even 200 bases long, or from about 6 to about 50 bases, for example about 10-25 bases, such as 12, 15 or 20 bases.

**Open reading frame:** A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide.

**Parenteral:** Administered outside of the intestine, for example, not via the alimentary tract. Generally, parenteral formulations are those that will be administered other than through ingestion or rectal administration. Parenteral especially refers to injections, whether administered intravenously, intrathecally, intramuscularly, intraperitoneally, intra-articularly, or subcutaneously, and various surface applications including intranasal, intradermal, and topical application, for instance.

**Pharmaceutical agent:** A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject or a cell.

**Pharmaceutically acceptable carriers:** The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington’s Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of therapeutics herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol
or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

**Preventing or treating a disease:** "Preventing" a disease refers to inhibiting the full development of a disease, for example in a person who is known to have a predisposition to a disease such as an autoimmune disorder. An example of a person with a known predisposition is someone with a history of diabetes in the family, or who has been exposed to factors that predispose the subject to a condition, such as lupus or autoimmune hepatitis. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom (including laboratory or tissue evidence) of a disease or pathological condition after it has begun to develop.

**Polypeptide:** A polymer in which the monomers are amino acid residues that are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The term polypeptide or protein as used herein encompasses any amino acid sequence and includes modified sequences such as glycoproteins. The term polypeptide is specifically intended to cover naturally occurring proteins, as well as those that are recombinantly or synthetically produced.

The term polypeptide fragment refers to a portion of a polypeptide that exhibits at least one useful epitope. The phrase “functional fragments of a polypeptide” refers to all fragments of a polypeptide that retain an activity, or a measurable portion of an activity, of the polypeptide from which the fragment is derived. Fragments, for example, can vary in size from a polypeptide fragment as small as an epitope capable of binding an antibody molecule to a large polypeptide capable of participating in the
characteristic induction or programming of phenotypic changes within a cell. The term soluble refers to a form of a polypeptide that is not inserted into a cell membrane.

**Protein:** A biological molecule expressed by a gene and comprised of amino acids.

**Purified:** In a more pure form than is found in nature. The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein preparation is one in which the protein referred to is more pure than the protein in its natural environment within a cell.

The term "substantially purified" refers to a molecule (for example, a nucleic acid, polypeptide, oligonucleotide, etc.) that is substantially free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated. In one embodiment, the molecule is a polypeptide that is at least 50% free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated. In another embodiment, the polypeptide is at least 80% free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated. In yet other embodiments, the polypeptide is at least 90% or at least 95% free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated.

**Quantify:** To determine the number, degree, or amount of something. The term encompasses both determining a numerical quantity and a relative amount.

**Separation moiety:** Any moiety that permits separation or depletion of a specific population of cells. For example, if depletion of specific T cells is desired, a specific binding complex is conjugated to a separation moiety, which permits selective depletion of specifically bound cells. Suitable separation moieties include any moiety that permits specific separation or depletion of cells, for instance by flow cytometric sorting or by targeting with specific antibodies coupled to magnetic beads, which are then removed with a magnet. One specific, non-limiting example of a suitable separation moiety is phycoerythrin.

**Sequence identity:** The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences,
otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or orthologs of the pestivirus replicon construct protein, and the corresponding cDNA sequence, will possess a relatively high degree of sequence identity when aligned using standard methods. This homology will be more significant when the orthologous proteins or cDNAs are derived from species that are more closely related (for example, human and chimpanzee sequences), compared to species more distantly related (for example, human and C. elegans sequences).


For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment is performed using the Blast 2 sequences function, employing the PAM30 matrix set to default
parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to a reference sequence will show increasing percentage identities when assessed by this method, such as at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., J. Mol. Biol., 215: 403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. The Search Tool can be accessed at the NCBI website, together with a description of how to determine sequence identity using this program. BLAST searching permits the determination of the sequence identity between a given sequence, for example a nucleotide sequence and a reference sequence. Nucleotide sequences with even greater similarity to a reference sequence will show increasing percentage identities when assessed by this method, such as at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity.

An alternative indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence remains hybridized to a perfectly matched probe or complementary strand. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, CSHL, New York, and Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes Part I, Chapter 2, Elsevier, New York. Nucleic acid molecules that hybridize under stringent conditions to a pestivirus replicon encoding sequence will typically hybridize to a probe based on
either an entire human pestivirus replicon encoding sequence or selected portions of the
gene under wash conditions of 2x SSC at 50 °C.

Nucleic acid sequences that do not show a high degree of identity can
nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic
code. It is understood that changes in nucleic acid sequence can be made using this
degeneracy to produce multiple nucleic acid molecules that all encode substantially the
same protein.

Subject: Living multi-cellular vertebrate organisms, a category that includes
both human and non-human mammals.

T cell: A white blood cell involved in the immune response. T cells include,
but are not limited to, CD4+ T cells and CD8+ T cells. A CD4+ T lymphocyte is an
immune cell that carries a marker on its surface known as “cluster of differentiation 4”
(CD4). These cells, also known as helper T cells, help orchestrate the immune
response, including antibody responses as well as killer T cell responses. CD8+ T cells
carry the “cluster of differentiation 8” (CD8) marker. In one embodiment, CD8+ T cells
are cytotoxic T lymphocytes. In another embodiment, CD8+ T cells are
IFN-gamma-producing T cells.

T cell response: A response of a T cell to a stimulus. In one embodiment, the
response is specific for a particular antigen (an “antigen-specific response”). In one
embodiment, a T cell response is a CD4+ T cell response or a CD8+ T cell response.

Therapeutic agent: Used in a generic sense, it includes treating agents,
prophylactic agents, and replacement agents. A particular example is an agent effective
to treat an autoimmune disorder.

Therapeutically effective amount [of an agent]: A quantity of a specified
agent (such as a pharmaceutical compound or composition) sufficient to achieve a
desired effect in a subject being treated. For instance, this can be the amount of an APL,
antagonist, or multimeric MHC/peptide epitope necessary to slow or halt disease
progression in a subject with an autoimmune disorder, or a dose sufficient to cause
regression of a disease, or which is capable of relieving symptoms caused by a disease, such as pain or swelling.

An effective amount of an APL, antagonist, or multimeric MHC/peptide epitope can be administered systemically or locally. In addition, an effective amount of an APL, antagonist, or multimeric MHC/peptide epitope can be administered in a single dose, or in several doses, for example daily, or multiple times daily, during a course of treatment. In some examples, administration of an agent is repeated daily, weekly, or monthly for at least 3, at least 5, at least 10, or more administrations. However, the effective amount of the APL, antagonist, or multimeric MHC/peptide epitope will be dependent on the preparation applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the compound. For example, a therapeutically effective amount of an APL, antagonist, or multimeric MHC/peptide epitope can vary from about 0.01 mg/kg body weight to about 1 g/kg body weight in some embodiments, or from about 0.01 mg/kg to about 100 mg/kg of body weight, based on efficacy.

The APLs, antagonists, and multimeric MHC/peptide epitopes disclosed herein have equal applications in medical and veterinary settings. Therefore, the general terms “subject” and “subject being treated” are understood to include all animals, including humans or other simians, dogs, cats, mice, rats, horses, and cows.

**Toxin moiety:** Any moiety that permits ablation of specific cells. Suitable toxin moieties include any cytotoxic moiety that can be conjugated to a peptide or complex, for example ricin, abrin, diphtheria toxin, a maytansinoid, or cisplatin. Generally, the toxin moiety is conjugated by means of a cross-linker, and is administered so as to specifically eliminate the target cells without exerting significant toxicity against other cells. Specific, non-limiting examples of toxin conjugates are disclosed in U.S. Patent Nos. 5,208,020; 4,863,726; 4,916,213; and 5,165,923.

**Transfected:** A process by which a nucleic acid molecule is introduced into cell, for instance by molecular biology techniques, resulting in a transfected cell. As used herein, the term transfection encompasses all techniques by which a nucleic acid
molecule might be introduced into such a cell, including transduction with viral vectors, transfection with plasmid vectors, and introduction of DNA by electroporation, lipofection, and particle gun acceleration.

**Vector:** A nucleic acid molecule as introduced into a host cell, thereby producing a transfected host cell. Recombinant nucleic acid vectors are vectors having recombinant nucleic acid sequences. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Hence “comprising A or B” means including A or B, or including A and B. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

**IV. Overview of Several Embodiments**

Autoimmune hepatitis (AIH) is a chronic, progressive, organ-specific autoimmune disease that, in the absence of immunosuppressive therapy, may result in complete tissue destruction and cirrhosis. In spite of extensive studies over the last two decades, a correlation was not found between the titer of several known antibodies and disease severity. Therefore, autoantibodies are considered serological markers for
diagnosis and classification of AIH, but do not appear to contribute to disease pathogenesis.

Instead, genetic factors are involved in the pathogenesis of AIH and HLA-DRB1*0301 and HLA-DRB1*0401 have been identified as independent determinants of susceptibility to AIH (Czaja (1997) *Hepatology*; 25: 317-323). The presence of disease susceptibility genes HLA-DRB1*0301 and HLA-DRB1*0401, the upregulation of HLA-DR molecules in the inflamed liver, the observation that HLA-DR-expressing hepatocytes function as antigen-presenting cells and activate specific liver infiltrating T lymphocytes Herkel et al., *Hepatology* 2003;3:1079-85), and the correlation between the size of the intrahepatic T cell infiltrate and disease severity all suggests that HLA-DRB1*0301 and HLA-DRB1*0401 present autoantigenic peptides to liver-infiltrating T cells and thereby activate these T cells and perpetuate AIH (McFarlane (2002) *Semin Liver Dis*; 22:317-324; Wucherpfennig (1995) *J Exp Med*; 181: 1597-1601).

This concept has been proven for other DRB1*0301 and DRB1*0401-associated autoimmune diseases (Wen et al., *J Clin Invest* 2001; 107:871-880; Pennesi et al., *J. Clin. Invest.,* 111(8):1171-80, 2003). Given the foregoing, it would be desirable to identify those autoantigenic peptides that are targeted by liver-infiltrating T cells in AIH in order to allow quantification of autoantigen-specific T cells. This would allow the monitoring of disease severity and permit studies on effector function, as well as providing therapeutic means to modulate and/or abrogate the pathogenic T cell response in order to treat the disease. Despite the urgency of the need to identify the CD4+ T cell targets, the targets for the intrahepatic cellular immune response were still unknown prior to this disclosure.

A. **T Cell Epitopes Involved in AIH**

Described herein are immunodominant T cell epitopes identified using a small animal model transgenic for the principal disease susceptibility gene, HLA-DRB1*0301. SLA/LP (soluble liver antigen/liver-pancreas antigen), the major autoantigen in AIH, is not only targeted by the humoral (Wies et al., *Lancet* 2000;
355:1510-1515) but also by the cellular immune response, and the specific SLA/LP T cell epitopes described herein are endogenously processed from the native SLA/LP protein are recognized in a HLA-DRB1*0301-restricted manner.


Using a panel of C- and N-terminally truncated peptides derived from the immunogenic 20mer sequences, a series of isolated epitopes present in the SLA/LP sequence also were identified (see, e.g., FIGs. 5-7). Exemplary T cell-reactive SLA/LP epitopes include, for example, CFWRRFHIHGIR (SEQ ID NO: 1); IQQGARVGRID (SEQ ID NO: 3), LIQQGARVGRID (SEQ ID NO: 7); IQQGARVGRI (SEQ ID NO: 9); MHGQQGARVGRID (SEQ ID NO: 11), NRLDRCLKAVRKER (SEQ ID NO: 5), RLDRCRLKAVRKE (SEQ ID NO: 10); IIWPRIDQKSCFK (SEQ ID NO: 2), and EEMALKLDNVLLDITYQD (SEQ ID NO: 4). Other representative epitopes can be obtained by continued amino- and carboxy-terminal truncation of, at least, SEQ ID NO: 2 and SEQ ID NO: 4 as described below, e.g., in Example 5. Still other exemplary immunoreactive epitopes of SLA/LP shown in FIGs. 5-7 include SLP/LP residues 184-198, 186-198, 186-196, 372-386, 372-385, 372-384, and/or 373-385.

In some embodiments, a SLA/LP T cell epitope includes SEQ ID NOs: 1-5, 7, 9-11 and/or variants thereof. In other embodiments, a SLA/LP T cell epitope includes

 Whereas the target of the immune system is SLA/LP in type 3 autoimmune hepatitis, the target in type 2 autoimmune hepatitis is liver kidney microsomal antigen (LKM). T cell-reactive epitopes for LKM can be determined using the same methods described for SLA/LP. Briefly, to identify T cell targets, HLA-DRB1*0301-transgenic mice are immunized with human recombinant liver kidney microsomal antigen, and LKM-specific antibodies and T cells are analyzed with recombinant LKM and a set of LKM-overlapping peptides with an ELISA immunoassay, a proliferation assay, and an ELISpot assay, respectively. Specific isolated epitopes are then fine mapped with cloned T cell hybrids and truncated peptides and tested for MHC restriction.

 With the provision herein of the amino acid sequences of numerous SLA/LP T cell epitopes, variants of such epitopes are now enabled. One embodiment of a variant epitope is altered peptide ligands (APLs) (as defined previously). Other variant SLA/LP epitope embodiments include peptides that differ in amino acid sequence from a disclosed SLA/LP T cell epitope sequence, but that substantially retain at least one function of a wild-type epitope (e.g., binding and/or activation of SLA/LP-specific T cells). In some embodiments, SLA/LP variant epitopes include peptides that share at least 60% amino acid sequence identity with a SLA/LP T cell epitope sequence.
provided herein; for example, some SLA/LP variant epitopes will share at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% amino acid sequence identity with a disclosed SLA/LP T cell epitope (for example, SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10, or 11). Methods for determining sequence identity have been described elsewhere in this disclosure (see, e.g., definition of "sequence identity").

In some embodiments, SLA/LP variant epitopes involve the substitution of one or several amino acids for amino acids having similar biochemical properties (so-called conservative substitutions). Conservative amino acid substitutions are likely to have minimal impact on the activity of the resultant peptide/epitope. Further information about conservative substitutions can be found, for instance, in Ben Bassat et al. (J. Bacteriol., 169:751-757, 1987), O'Regan et al. (Gene, 77:237-251, 1989), Sahin-Toth et al. (Protein Sci., 3:240-247, 1994), Hochuli et al. (Bio/Technology, 6:1321-1325, 1988) and in widely used textbooks of genetics and molecular biology. The Blosum matrices are commonly used for determining the relatedness of polypeptide sequences. The Blosum matrices were created using a large database of trusted alignments (the BLOCKS database), in which pairwise sequence alignments related by less than some threshold percentage identity were counted (Henikoff et al., Proc. Natl. Acad. Sci. USA, 89:10915-10919, 1992). A threshold of 90% identity was used for the highly conserved target frequencies of the BLOSUM90 matrix. A threshold of 65% identity was used for the BLOSUM65 matrix. Scores of zero and above in the Blosum matrices are considered "conservative substitutions" at the percentage identity selected.

The following table shows exemplary conservative amino acid substitutions:

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Very Highly - Conserved Substitutions</th>
<th>Highly Conserved Substitutions (from the Blosum90 Matrix)</th>
<th>Conserved Substitutions (from the Blosum65 Matrix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Ser</td>
<td>Gly, Ser, Thr</td>
<td>Cys, Gly, Ser, Thr, Val</td>
</tr>
<tr>
<td>Arg</td>
<td>Lys</td>
<td>Gln, His, Lys</td>
<td>Asn, Gln, Glu, His, Lys</td>
</tr>
<tr>
<td>Asn</td>
<td>Gln, His</td>
<td>Asp, Gln, His, Lys, Ser, Thr</td>
<td>Arg, Asp, Gln, Glu, His, Lys, Ser, Thr</td>
</tr>
<tr>
<td>Asp</td>
<td>Glu</td>
<td>Asn, Glu</td>
<td>Asn, Gln, Glu, Ser</td>
</tr>
<tr>
<td>Cys</td>
<td>Ser</td>
<td>None</td>
<td>Ala</td>
</tr>
<tr>
<td>Gln</td>
<td>Asn</td>
<td>Arg, Asn, Glu, His, Lys, Met</td>
<td>Arg, Asn, Asp, Glu, His, Lys, Met, Ser</td>
</tr>
<tr>
<td>Glu</td>
<td>Asp</td>
<td>Asp, Gln, Lys</td>
<td>Arg, Asn, Asp, Gln, His, Lys, Ser</td>
</tr>
</tbody>
</table>
Table 5.

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Very Highly - Conserved Substitutions</th>
<th>Highly Conserved Substitutions (from the Blosum90 Matrix)</th>
<th>Conserved Substitutions (from the Blosum65 Matrix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>Pro</td>
<td>Ala</td>
<td>Ala, Ser</td>
</tr>
<tr>
<td>His</td>
<td>Asn; Gln</td>
<td>Arg, Asn, Gln, Tyr</td>
<td>Arg, Asn, Gln, Glu, Tyr</td>
</tr>
<tr>
<td>Ile</td>
<td>Leu; Val</td>
<td>Leu, Met, Val</td>
<td>Leu, Met, Phe, Val</td>
</tr>
<tr>
<td>Leu</td>
<td>Ile; Val</td>
<td>Ile, Met, Phe, Val</td>
<td>Ile, Met, Phe, Val</td>
</tr>
<tr>
<td>Lys</td>
<td>Arg; Glu</td>
<td>Arg, Asn, Gln, Glu</td>
<td>Arg, Asn, Gln, Glu, Ser</td>
</tr>
<tr>
<td>Met</td>
<td>Leu; Ile</td>
<td>Gln, Ile, Leu, Val</td>
<td>Gln, Ile, Leu, Phe, Val</td>
</tr>
<tr>
<td>Phe</td>
<td>Met; Leu; Tyr</td>
<td>Leu, Trp, Tyr</td>
<td>Ile, Leu, Met, Trp, Tyr</td>
</tr>
<tr>
<td>Ser</td>
<td>Thr</td>
<td>Ala, Asn, Thr</td>
<td>Ala, Asn, Asp, Gln, Glu, Glu, Gly, Lys, Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>Ser</td>
<td>Ala, Asn, Ser</td>
<td>Ala, Asn, Ser, Val</td>
</tr>
<tr>
<td>Trp</td>
<td>Tyr</td>
<td>Phe, Tyr</td>
<td>Phe, Tyr</td>
</tr>
<tr>
<td>Tyr</td>
<td>Trp; Phe</td>
<td>His, Phe, Trp</td>
<td>His, Phe, Trp</td>
</tr>
<tr>
<td>Val</td>
<td>Ile; Leu</td>
<td>Ile, Leu, Met</td>
<td>Ala, Ile, Leu, Met, Thr</td>
</tr>
</tbody>
</table>

In some examples, SLA/LP variant epitopes can have no more than about 1, 2, 3, 4, 5, 6, 8 or 10 conservative amino acid changes (such as, very highly conserved or highly conserved amino acid substitutions) as compared to SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10, or 11. In other examples, one or several hydrophobic residues (such as Leu, Ile, Val, Met, Phe, or Trp) in a SLA/LP variant epitope sequence can be replaced with a different hydrophobic residue (such as Leu, Ile, Val, Met, Phe, or Trp) to create a variant functionally similar to a disclosed SLA/LP variant epitope. In certain examples, substitutions are made in a peptide including the epitope, but are made in a portion of the peptide not including the epitope.

As demonstrated herein, a SLA/LP T cell epitope can vary by one to a few amino acids at the N-terminal or C-terminal end without substantial loss of function. Accordingly, variant SLA/LP T cell epitopes are envisioned to include a disclosed SLA/LP T cell epitope or immunogenic peptide that lacks one or more amino acid residues from its C- and/or N-terminus while substantially retaining the function of the epitope from which the variant derives (e.g., reactivity with a SLA/LP-specific T cell when presented by an appropriate MHC). In one embodiment, a variant SLA/LP T cell epitope is a disclosed SLA/LP T cell epitope or SLA/LP immunogenic peptide (e.g., SEQ ID NO: 1-5, 7, or 9-11, or SLA/LP (SEQ ID NO: 6) residues 1-20, 9-28,

The peptides and variant peptides described herein can be synthesized by any standard chemical means or by any in vitro recombinant DNA technique known in the art. Methods well known to those skilled in the art can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, N.Y., 1989), and Ausubel et al., Current Protocols in Molecular Biology, (Green Publishing Associates and Wiley Interscience, N.Y., 1989). Exemplary techniques for peptide are also described by, for instance, Bodanszky and Bodanszky,

**B. Methods of Detection**

Because the quantity (e.g., number) and function of T cells specific for the epitopes described herein reflects the severity of the AIH, the quantity and function of these cells can be determined in order to monitor disease progression. For example, not only can the presence of activated T cells specific for the epitopes described herein be used as a diagnostic test for AIH, but their quantity and function in a particular subject also can be studied and compared with a baseline level, for example a level previously determined in the same subject or a level established by comparison to one or more other subjects, for example a population of subjects with AIH or a population of subjects without AIH. Generally speaking, an increase in the level of activated, functional T cells specific for the disclosed epitopes is indicative of a worsening of disease, and a decrease indicates an improvement in the disease, for example a disease remission.

In some embodiments, the level or function of activated T cells specific for the disclosed epitopes is used as a measure of the general extent of disease in a particular subject. In other embodiments, the level is compared to previous levels in order to make treatment decisions or evaluate the efficacy of a treatment, for example treatment with an anti-inflammatory drug such as prednisone, azathioprine, mycophenylate mofetil, cyclosporine or tacrolimus. The level or function of activated T cells specific for the disclosed epitopes also can be used to evaluate any of the therapeutic approaches described below.
The level of activated T cells specific for the disclosed epitopes can be quantified by any method known to one of skill in the art, including but not limited to ELISpot assays and multimer MHC/peptide epitope assays, both of which are described below. The function of activated T cells specific for the disclosed epitopes can be studied by any method known to one of skill in the art, including but not limited to staining the cells for intracellular or secreted cytokines, determining their level of proliferation or cytotoxicity, or determining their functional interaction with other cells, such as B cells, CD8 cell or dendritic cells.

1. ELISpot Assay

A cytokine ELISpot assay is designed to enumerate cytokine secreting cells in single cell suspensions of lymphoid tissue, liver tissue, bone marrow or preparations of peripheral blood mononuclear cells (PBMC). The assay has the advantage of detecting only activated/memory T cells and the cytokine release can be detected at the single cell level, allowing direct determination of T cell frequencies. This permits a direct enumeration of peptide-reactive T cells without prior in vitro expansion, making the ELISpot assay suitable for monitoring T cell responses.

The cytokine ELISpot is based on the method originally described by Sedwick and Holt for the detection of single B cells secreting antibody, but has been modified to detect cells that release cytokines such as GM-CSF, IFN-gamma, IL-2, IL-4, IL-5, IL-10, IL-13 and TNF-alpha or other molecules such as granzymes and is commonly called an ELISpot assay. The higher sensitivity of ELISpot in comparison to that of ELISA or intracellular staining is due to the plate-bound antibodies directly capturing the cytokine secreted around the cell before it is diluted in the supernatant, trapped by high-affinity receptors or degraded by proteases.

An exemplary detailed ELISpot assay protocol is presented below in Example 1. Briefly, in one specific, non-limiting example, for ex vivo analysis of immune responses of human subjects ELISpot plates are coated with a suitable antibody, for instance purified anti-human interleukin-2 (IL-2) monoclonal antibody or an anti- GM-CSF, IFN-gamma, IL-4, IL-5, IL-10, IL-13, TNF-alpha, or granzyme antibody, incubated,
washed, blocked with PBS/1% bovine serum albumin (BSA), washed, and blocked with complete culture medium. Recombinant SLA/LP, LKM, or synthetic peptides are used to stimulate T cells from a subject, for example cells isolated from blood, lymph nodes, or liver tissue, and controls include both a negative (no peptide) and positive (phytohemagglutinin, or common recall antigens such as tetanus toxoid) control. After incubation, the plates are washed and incubated with labeled (for example, biotin-labeled) anti-human IL-2 antibody, washed, and visualized (for example, with avidin-enzyme complex and substrate solution). Spots are evaluated and counted with an ELISpot reader. The number of specific spots is obtained by subtracting the mean number of spots in negative control wells from the mean number of spots in experimental wells.

2. **MHC/peptide Epitope Multimers**

Alternate methods useful for quantifying activated T cells specific for the epitopes disclosed herein are described, for instance, in U.S. Patent No. 5,635,363 or 5,869,270, and/or 6,232,445.

In one example, a stable multimeric (for example dimeric, trimeric, tetrameric, or pentameric) complex of major histocompatibility complex (MHC) protein subunits and any of the epitopes or immunogenic peptides of the present disclosure is prepared by methods known in the art (see, for example, U.S. Patent No. 5,635,363 or 6,232,445, or Day et al., *J. Clin. Invest.*, 112(6):831-842, 2003) or is obtained from a commercial supplier (e.g., Beckman Coulter (Brea, CA or Miami, FL). The specificity of the multimeric binding complex is determined both by the antigenic peptide and the MHC protein. The binding complex binds stably with the antigen receptor on the surface of T cells, allowing the detection of antigen specific T cells. This binding complex is useful for detection, quantification, characterization, and separation of specific T cells.

In another example, a single chain (sc) MHC class II molecule is combined with any of the epitopes of the present disclosure (see, for example, U.S. Patent No. 5,869,270). The scMHC-epitope complex binds stably with the antigen receptor on the surface of T cells, allowing the detection of antigen specific T cells. This binding
complex is useful for detection, quantification, characterization, and separation of specific T cells.

The T cell receptor (TCR) specificity determines what antigens will activate that particular T cell. Usually, T helper cells express CD4 on their surface, and are activated by binding to a complex of antigenic peptide and Class II MHC molecule. Generally, cytolytic T cells express CD8 on their surface, and are activated by binding to a complex of antigenic peptide and Class I MHC molecule. The specificity of the T cell antigen receptor is the combination of peptide and MHC molecule that binds to that particular TCR with sufficient affinity to activate the T cell. Therefore, multimers of class I MHC molecules usually are used to detect CD8+ T cells, and class II multimers usually are used to detect CD4+ T cells.

The T cells may be from any source, for example a physiologic sample, such as blood or lymph, but samples may also be derived from liver tissue. The binding complex is added to a suspension comprising T cells of interest, and incubated at about 4°C for a period of time sufficient to bind the available cell surface receptor.

Where a second stage labeling reagent is used, the cell suspension is washed and resuspended in medium prior to incubation with the second stage reagent. Alternatively, the second stage reagent may be added directly into the reaction mix.

Detecting and/or quantifying specific T cells in a sample is accomplished using one of a variety of specific assays. In general, the assay measures the binding between a patient sample, usually blood derived, and generally in the form of plasma or serum, and the multimeric binding complexes. For instance, a sandwich assay is performed by first attaching the multimeric binding complex to an insoluble surface or support, for instance beads, membranes, or microtiter plates. Before adding patient samples or fractions thereof, the non-specific binding sites on the insoluble support are blocked with bovine serum albumin, casein, gelatin, or a similar non-reactive protein.

The sample is then added to the support and incubated sufficiently for the T cells to bind the insoluble binding complex. After incubation, the insoluble support is washed, and a solution containing specific second receptor is applied. The receptor may
be any compound that binds patient T cells with sufficient specificity such that they can be distinguished from other components present, for example an antibody specific for a common T cell antigen, for instance anti-thy-1 or anti-CD45. The T cell specific antibody may be labeled to facilitate direct or indirect quantification of binding. For instance, labels that permit direct measurement include but are not limited to radiolabels, dyes, beads, chemiluminescers, colloidal particles, and the like. Examples of labels that permit indirect measurement of binding include enzymes where the substrate may provide for a colored or fluorescent product, for instance horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like.

After the second receptor or second receptor-conjugate has bound, the insoluble support is again washed, and the signal produced by the bound conjugate is detected by conventional means. The number of bound T cells detected will be compared to control samples.

C. Methods of Treatment

In addition to monitoring disease progression in AIH, the AIH-specific T cell epitopes described herein also can be used to treat AIH. Several specific, non-limiting methods of using the peptides to treat AIH are described below. In general, the methods are used to treat or prevent the onset of autoimmune hepatitis in a susceptible individual, for example, one in which anti-SLA/LP or LKM antigens have been detected, or one in which symptoms are present. The methods are particularly useful in treating subjects with AIH for whom conventional therapies such as immunosuppression are insufficient, or for whom the side effects of that therapy are intolerable.

1. Therapeutic Approaches Involving Altered Peptide Ligands (APLs) or Antagonists

APLs are variant peptide epitopes in which at least one of the amino acid residues of the epitope are substituted with different amino acid residues, yet the APLs still bind to the same MHC molecules as the wild-type epitopes and activate T cells involved in a disease process. For example, in some embodiments from about one to
about four amino acid residues are different from the corresponding residues of the wild-type epitope. Methods of therapy and prophylaxis using APLs are described in U.S. Patent No. 6,562,943.

An APL activates a response in CD4+ T cells that is qualitatively different from that activated by the parent peptide. For example, while the parent peptide may activate a helper T cell 1- (Th1-) type response in which the cytokines IL-2, IFN-gamma, and TNF-alpha are produced by the activated CD4+ T cells, an APL derived from this parent peptide might instead activate a helper T cell 2- (Th2-) type response in the CD4+ T cells. In a Th2 response, the cytokines IL-4, IL-5, and IL-10 are produced by the activated CD4+ T cells. Alternatively, if a particular parent peptide elicits a Th2 response in a given CD4+ T cell, an APL derived from the parent peptide could activate a Th1 response in the T cell. In general, Th1 responses are associated with cell-mediated immune responses and Th2 responses are associated with antibody-mediated immune responses.

The amino acid substitutions in an APL or antagonist can be radical. For example, an amino acid with a positively charged side chain (for example, lysine) can be replaced by an amino acid with a negatively charged side chain (for example, aspartic acid) or a hydrophobic side chain (for example, isoleucine) and vice versa. In addition, an amino acid with a bulky side chain (for example, tryptophan) can be replaced with an amino acid with small side chain (for example, glycine or alanine) and vice versa. Alternatively, the substitutions can be conservative. For example, a negatively charged amino acid can be replaced with another negatively charged amino acid (for example, aspartic acid with glutamic acid) or one hydrophobic amino acid with another hydrophobic amino acid (for example, leucine with valine or isoleucine).

An APL that has the ability to elicit a cytokine response in CD4+ T cells that is non-pathogenic and/or is suppressive of a pathogenic CD4+ T cell cytokine response elicited by the APL's parental peptide is useful in treating a disease caused by the pathogenic CD4+ T lymphocyte response to the parental peptide, for example a
pathogenic response to one of the epitopes disclosed herein. Treatment can be either in
vivo or in vitro, as described below.

Antagonists include peptide and non-peptide ligands that bind to the same
T cells as the specific epitopes, but block (antagonize) its function.

In one in vivo approach, the APL or antagonist is administered to the subject by
any parenteral route, for example intravenously, intra-arterially, or delivered directly to
an appropriate tissue (for example, spleen, lymph node, or liver). The dosage required
depends on the choice of APL or antagonist, the route of administration, the nature of
the formulation, the nature of the patient's illness, and the judgment of the attending
physician, but suitable dosages are in the range of 0.1-100.0 µg/kg.

2. **Therapeutic Approaches Involving MHC/peptide Multimers**

There are several ways to use the T cell isolation techniques described above in
section entitled “MHC/peptide Multimers” to treat AIH. For example, immune cells
may be isolated from a subject and selectively depleted of T cells reactive with one of
the epitopes in order to lessen the pathological autoimmune response found in AIH.
Several of these methods are described below.

Inhibition or modification of immune function may be achieved by ablation or
depletion of specific epitope-reactive T cells. As described in greater detail in US
Patent No. 5,635,363, the multimeric binding complexes allow a therapy to be targeted
to very specific subsets of T cells, for example the subset of T cells that are activated by
the peptides disclosed herein.

If ablation of specific T cells is desired, the multimeric binding complex can be
conjugated to a toxin moiety. Suitable toxin moieties include any cytotoxic moiety that
can be conjugated to the multimeric complex, for example ricin, abrin, diphtheria toxin,
a maytansinoid, or cisplatin. Generally, the toxin moiety is conjugated to the binding
complex by means of a cross-linker, and is administered so as to specifically eliminate
the target T cells without exerting significant toxicity against other cells. Specific,
non-limiting examples of toxin conjugates are disclosed in U.S. Patent Nos. 5,208,020;
4,863,726; 4,916,213; and 5,165,923.
If depletion of specific T cells is desired, the multimeric binding complex is conjugated to a separation moiety. Suitable separation moieties include any moiety that allows removal of the specifically-bound T cells, for instance by flow cytometric sorting or by targeting with specific antibodies coupled to magnetic beads, which are then removed with a magnet. One specific, non-limiting example of a suitable separation moiety is phycoerythrin.

The appropriate dose of binding complex is determined by measuring the effect of the binding complexes on signs of clinical improvement, for example a reduction in liver enzymes. The binding complexes will normally be administered parenterally, for example intravenously, intramuscularly, subcutaneously, or intra-arterially. In some embodiments, the dose of the binding complexes is repeated periodically, for example daily, weekly, or monthly.

When administered parenterally, the binding complexes are formulated in an injectable dosage form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and Hanks' solution. Non-aqueous vehicles such as fixed oils and ethyl oleate may also be used. The vehicle may contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability, for instance buffers and preservatives. Suitable pharmaceutical vehicles and their formulations are described in Remington's Pharmaceutical Sciences, by E. W. Martin.

The binding complexes described herein also can be used therapeutically ex vivo. One embodiment involves isolating immune cells from the subject and ablating T cells reactive with one of the immunogenic region peptides described above using a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex in which the peptide antigen includes one of the immunogenic region peptides described above. The multimeric MHC/peptide antigen complex is conjugated to a toxin moiety or a separation moiety as described above. After removal of the specific T cells that bind to the multimeric MHC/peptide antigen complex, the remaining cells are then reintroduced into the subject.
EXAMPLES

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

REPRESENTATIVE METHODS AND MATERIALS

FIG. 1 provides a schematic representation of exemplary methods used in the following examples. Briefly, the SLA/LP autoantigen (Wies et al., Lancet, 355:1510-5, 2000) was used to immunize 'humanized' mice, which are transgenic for HLA-DRB1*0301 and do not express murine MHC class II (Kong et al., J. Exp. Med., 184:1167-72, 1996). Immunization of transgenic mice generated SLA/LP-specific autoantibodies and murine T cells that recognized SLA/LP in the context of the human DRB1*0301. These T cells and hybridomas derived therefrom were used to map the immunogenic peptides within SLA/LP and to identify optimal DR3-restricted sequences (epitopes) in those peptides. Fluorescently labeled tetrameric complexes of DRB1*0301, which present an SLA/LP epitope of interest (DRB1*0301/epitope tetramers), were custom-made to detect autoantigen-specific T cells ex vivo. This Example provides exemplary materials and methods for obtaining the foregoing results.

A. Synthetic Peptides and Antibodies

A set of 20-mer peptides overlapping by 12 amino acids and spanning amino acid residues 1-422 of human SLA/LP was synthesized at Mimotopes (Clayton, Australia). Shorter SLA/LP peptides and the DRB1*0301-restricted hepatitis C virus (HCV) peptide HCV261-269 (VLVLNPSVA; SEQ ID NO: 8) (Diepolder et al., J. Virol., 71:6011-6019, 1997) were synthesized at the Facility for Biotechnology Resources, Center for Biologics Evaluation and Research, Food and Drug Administration (Bethesda, MD). Peptides were resuspended at 20 mg/ml in dimethyl sulfoxide (DMSO) and diluted with phosphate-buffered saline solution (PBS) to 2 mg/ml.
Tetramers DRB1*0301/SLA\textsubscript{184-198} and DRB1*0301/SLA\textsubscript{373-386} were custom-made by Beckman Coulter (Fullerton, CA).

Anti-mouse CD4-PE or -FITC (clone 129.19), CD3e-APC-Cy7 (clone 145-2C11), CD19-PE (clone 1D3), CD11a-PE-Cy7 (2D7), CD62L-APC (clone MEL-14) and anti-human CD3-APC (clone UCHT1), CD4-PE-Cy7 (clone SK3), CD25-APC-Cy7 (clone M-A 251), CD8-PE-Cy5 (clone RPA T-8), CD16-PE-Cy5 (clone 3G8), HLA-DR-FITC (clone L243) and CD19-PE-Cy5 (clone HIB 19) as well as purified antibodies to I-A/I-E (clone 2G9) and to HLA-DR, -DP, -DQ (clone TÜ39) were purchased from BDPharmingen (San Diego, CA). Anti-human CD14-PE-Cy5 (clone TÜK4) was purchased from Serotec (Raleigh, NC).

B. Cloning and Purification of Recombinant, Human SLA/LP Protein

Recombinant human SLA/LP antigen (Accession AAD33963) was synthesized and purified as previously described (Wies et al. (2000) Lancet; 355:1510-1515). SLA/LP protein was purified on nickel-nitrilo-triacetic acid coupled agarose with the Qia express system (Qiagen). Purified protein was electrophoretically separated on a 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE), blotted, and tested for its reactivity with serum positive for SLA/LP autoantibodies and control serum samples.

C. Mouse Immunization

HLA-DRB1*0301-transgenic mice, that do not express endogenous class II at detectable level (DR3.AB0) (Kong et al., J. Exp. Med., 184:1167-72, 1996) were bred and maintained in a clean conventional facility. C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Experiments were approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

For epitope mapping studies, eight to twelve week-old mice were immunized with (i) 40 μg of human recombinant SLA/LP or (ii) 50 μg of peptides SLA\textsubscript{81-100}, SLA\textsubscript{89-108} and SLA\textsubscript{97-116} or (iii) 50 μg of peptides SLA\textsubscript{177-196} and SLA\textsubscript{185-204} or (iv) 50 μg of peptides SLA\textsubscript{403-416} and SLA\textsubscript{408-418}, in 100-200 μl PBS and emulsified with an equal amount of AS01B adjuvant (GlaxoSmithKline, Rixensart, Belgium) (Vandepapeliere et
al., Vaccine, 23:2591-601, 2005) at the base of the tail and both hind foot pads. Mice were reimmunized after 3 weeks and killed after additional 3 weeks or reimmunized after four weeks and killed 10 days after the last immunization. Serum samples were collected as indicated. Lymphocytes from draining lymph nodes and spleen were isolated by either mechanical disruption or enzymatic digestion (400 µg/ml Liberase CI (Roche Diagnostics, Indianapolis, IN) of the respective organs, followed by filtration of the cell suspension through a 70 µm nylon cell strainer (BD Falcon, Bedford, MA) and lysis of red blood cells with ACK lysing buffer (Bio-Whittaker, Walkersville, MD).

A protocol for inducing T cell mediated, autoimmune diabetes (Lang et al., Nat. Med., 11:138-45, 2005) was adapted to induce AIH. Briefly, mice were immunized with 1 mg of peptides SLA185-204 and SLA373-386, respectively, in 200 µl PBS s.c. at the base of tail on days 0, 2, 5, 9, 20 and 40. In addition, mice were injected with 50 µg CpG ODN 1826 (Coley Pharmaceutical Canada, Ottawa, Canada) s.c. at the base of tail on day 0, 2 and 5, 10^4 units recombinant mouse IFN-α (Cell Sciences, Canton, MA) i. v. on day 12 and 200 µg poly(I:C) (Fluka Sigma-Aldrich, St. Louis, MO) and 20 µg lpxoribine (Invivogen, San Diego, CA) on days 14 and 20 as previously described (Lang et al., Nat. Med., 11:138-45, 2005). Blood samples for ALT measurement were drawn on days 25, 29, 35 and 40. Ten days after the last immunization, mice were sacrificed.

D. Analysis of the Humoral Immune Response

SLA/LP-specific antibodies were detected with the QUANTA Lite™ SLA/LP EIA kit (Inova Diagnostics, San Diego, CA) according to the manufacturer’s instructions except for the use of an HRP-labeled polyclonal goat anti-mouse Ig (BD Pharmingen, dilution 1:2000) as detection antibody. Isotyping was performed with a mouse immunoglobulin screening/isotyping kit (Zymed Laboratories, San Francisco, CA). Fine mapping of the antibody specificity was performed by EIA and 1/100 dilutions of sera using microtiter plates (Falcon) coated with SLA/LP-overlapping peptides (10 µg/ml).
E. Analysis of the Cellular Immune Response of Immunized Mice

1. Proliferation Assays

Quadruplicate cultures of $5 \times 10^5$ spleen cells of immunized mice were stimulated for 3 days with or without human recombinant SLA/LP at the indicated concentration or 10 ng/ml phorbol myristic acetate (PMA) / 1 µM ionomycin (both Sigma-Aldrich) in 96-well round bottom plates in standard RPMI 1640 cell culture medium containing 10% heat-inactivated FBS (Bio-Whittacker, Walkersville, MD), 50 µM β-mercaptoethanol (Gibco), 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 10 mM HEPES, and 1 mM sodium pyruvate (Cellgro, Herndon, VA). For the last 16 h, 1 µCi $^3$H-thymidine (ICN, Costa Mesa, CA) was added, cells were harvested 16 h later with a Packard Filtermate 96-well-harvester (Perkin Elmer, Wellesley, MA) and incorporated radioactivity was measured with a Packard Topcount (Perkin Elmer).

2. Enzyme-linked Immunospot (ELISpot) Assays

Ex vivo ELISpot assays were performed as described (Wedemeyer et al., Gastroenterology, 121:1158-66, 2001) with provided modifications. Briefly, ELISpot plates (Millipore, Bedford, MA) were coated with purified anti-mouse interleukin-2 (IL-2) monoclonal antibody (2 µg/ml in 100 µl PBS, clone JES6-1A12, PharMingen) at 4°C overnight. After four washing steps with PBS, plates were blocked with PBS/1% bovine serum albumin (BSA) for two hours at room temperature, washed two more times with PBS and blocked with complete culture medium for 30 minutes at room temperature. Recombinant SLA/LP (2.5 µg/ml) or synthetic peptides (10 µg/ml) were used to stimulate $5 \times 10^5$ splenocytes/well in 200 µl complete culture medium. All cultures were set up in triplicates with appropriate negative (no peptide) and positive (10 ng PMA/1 µM ionomycin) controls. After 24 hours, plates were washed three times with PBS and four times with PBS/0.05% Tween-20 and incubated with 2 µg/ml biotin-conjugated anti-mouse IL-2 antibody (clone JES6-SH4, PharMingen) in 100 µl PBS/1% BSA/0.05% Tween-20 at 4°C overnight. The next day, plates were washed four times with PBS/0.05% Tween-20 and 100 µl of avidin-enzyme complex (Vector
Laboratories, San Francisco, CA) was added. After incubation for one hour at room temperature, plates were washed four times with PBS and 100 μl 3-amino-9-ethylcarbazole (AEC) substrate solution (Sigma-Aldrich, St. Louis, MO) was added. After approximately four minutes, the reaction was stopped under tap water. Spots were evaluated and counted with a KS ELISpot reader (Carl Zeiss Inc., Thornwood, NY)

3. **Tetramer Analysis**

CD4⁺ T cells were negatively isolated from spleen and lymph node cells with magnetic beads over MACS columns (Miltenyi Biotec, Auburn, CA), incubated for 10 min with antiCD16/antiCD32 to block Fc receptors (clone 2.4G2, BD Pharmingen), washed and stained with 2.5 μg/ml ethidium bromide monoazide (EMA) (Sigma-Aldrich) under fluorescent light for 10 min. Cells were washed again, stained with 10 μl of either DRB1*0301/SLA₁₈₄₋₁₉₈-tetramer or DRB1*0301/SLA₃₇₃₋₃₈₆-tetramer under addition of unconjugated anti-mouse TCR-β (clone H57-597, eBiosciences, San Diego, CA) at 37°C for 3h. Ten μl of each DRB1*0301/epitope-tetramer was determined to be the optimal amount in titration experiments. Antibodies against cell surface molecules were added for the last 30 min. Cells were washed and immediately analyzed on an LSRII flow cytometer (BD Biosciences, San Jose, CA) using FACS Diva (BD Biosciences, San Jose, CA) and FlowJo (TreeStar, San Carlos, CA) software. EMA-positive cells were excluded and CD3⁺CD4⁺ T cells were analyzed for DRB1*0301/epitope-tetramer binding and expression of CD62L and CD11a.

4. **Generation of T Cell Hybridomas**

Sixty million spleen cells of SLA/LP-immunized mice were stimulated with 5 μg/ml of SLA/LP or 10 μg/ml of the immunization peptides in 5 ml culture medium. After three days, cells were washed with PBS and resuspended in fusion medium (culture medium without FCS). The fusion partner cell line was BWZ.36 (kindly provided by Nilabh Shastri, University of California, Berkeley, CA), a TCRalpha/beta-negative cell line transfected with the β-galactosidase (LacZ) reporter gene under the control of the NFAT enhancer element of the IL-2 gene. BWZ.36 were maintained in complete culture medium with 1 mg/ml hygromycin B (Invitrogen,
Carlsbad, CA), washed with PBS and resuspended in fusion medium without hygromycin B.

Cell fusion was performed as described (Malarkannan et al., Meth. Mol. Biol., 156:265-72, 2001). Briefly, 2x10^7 spleen cells were mixed with 4x10^7 BWZ.36 cells in a 50 ml conical tube, centrifuged, and the supernatant was taken off completely. The cells were incubated with 1 ml of 37°C-warm 50% polyethylene glycol PEG1500 (Roche Diagnostics, Indianapolis, IN) for 1–2 minutes while constantly stirring, followed by slow addition of 10 ml 37°C fusion medium. Cells were centrifuged at 1200 rpm for five minutes and resuspended at a concentration of 3x10^5 T cells per ml complete medium. Fused cells were plated out at 3x10^4/well in 96-well flat-bottom plates and incubated overnight at 37°C, 5% CO₂. The next day, 100 μl selection medium was added, consisting of complete medium supplemented with 2x Aminopterin, 2x Hypoxanthine, 2x Thymidine (HAT, Gibco, Carlsbad, CA), and 2 mg/ml hygromycin B (Invitrogen, Carlsbad, CA). Colonies of growing hybrids were usually visible after 8-10 days and screened for their antigen-specificity in a T-cell activation assay (see below). Positive colonies were cultured in drug withdrawal medium (complete medium, supplemented with 1x hypoxanthine, 1x thymidine (HT, Gibco), and 1 mg/ml hygromycin B for ten days. Established hybrids were maintained in complete medium, supplemented with 0.5 mg/ml hygromycin B and further subcloned by limiting dilution at 0.3 cells/well.

5. Hybrid Activation and LacZ Assays

For the screening of antigen-specific hybrids and for the mapping of optimal T cell epitopes (e.g., those epitopes having the fewest amino acid residues and the highest relative activity compared to related epitopes), duplicate or triplicate cultures of 0.2–1x10^5 T cell hybridomas were stimulated overnight with 2x10^5 autologous, irradiated (3,000 rad) spleen cells with or without SLA/LP peptides at the indicated concentrations and with or without 2.5 or 10 μg anti I-A / anti I-E (clone 2G9, BD Pharmingen) or anti-HLA-DR, -DP, -DQ (TÜ39, BD Pharmingen) or isotype control antibodies. TCR-activation-induced transcription of the lacZ reporter gene via
activation of the NFAT enhancer element of the IL-2 gene. To measure LacZ activity, cultures were washed extensively and lysed with 100 μl buffer (1 mM MgCl₂, 0.125% NP-40 (Calbiochem, La Jolla, CA) and 0.15 mM chlorophenol red β-galactoside (CPRG, Roche) in PBS). After 8h at 37°C, the reaction was stopped with 100 μl buffer (300 mM glycine and 15 mM Na₂-EDTA in water, pH 12). Absorption was read at 575 nm with a 96-well Benchmark Microplate reader (Bio-Rad, Hercules, CA) with 620 nm as reference wavelength.

F. Detection of SLA/LP-specific CD4⁺ T Cells in Humans

PBMC were obtained from a patient with anti-SLA/LP-positive AIH, followed at the University of Mainz, Germany and a patient with a past history of hepatitis B (anti-HBs-positive, anti-HBe-negative, HB-Ag negative, HBeAg negative) followed at the NIDDK under protocols approved by the respective Ethics Committee and Institutional Review Board. Both patients were HLA-DRB1*0301-positive, tested negative for HCV and provided written informed consent. Ten million PBMC were stained with 1 μM carboxyfluorescein diacetate succinimidylester (CFSE, Molecular Probes, Eugene, Oregon) in 2 ml PBS for 5 min at room temperature. After addition of 5 ml FCS and three washing steps in PBS, cells were stimulated for 10 days with 10 μg/ml of the DRB1*0301-restricted SLA₃₇₇-₃₈₆ peptide or the DRB1*0301-restricted HCV₆₂₁-₆₂₉ control peptide (Diepolder et al., J. Virol., 71:6011-6019, 1997), respectively. Forty U/ml IL2 were added on days 4 and 7. On day 10, cells were stained with EMA, the respective DRB1*0301/epitope-tetramer and antibodies against cell surface proteins and immediately analyzed by flow cytometry. After EMA/CD8/CD14/CD16/CD19 exclusion gating, the CD3⁺CD4⁺ lymphocyte population was analyzed for tetramer binding and CFSE content.

E. Statistical analysis

A Mann-Whitney U test was used to assess differences in the strength of immune responses. A p-value of <0.05 was considered statistically significant.
Example 2

HUMORAL IMMUNE RESPONSE TO HUMAN RECOMBINANT SLA/LP IN HLA-DRB1*0301 TRANSGENIC MICE

This Example demonstrates that HLA-DRB1*0301-transgenic, but not nontransgenic mice, generate SLA/LP-specific autoantibodies of the same specificity as in AIH in humans.

DRB1*0301-transgenic mice were subcutaneously immunized with human recombinant SLA/LP and AS01B adjuvant (Vandepapeliere et al., Vaccine, 23:2591-601, 2005). As shown in FIG. 2A, a humoral immune response against SLA/LP was detectable after a single immunization and increased further after a booster immunization. Isotyping revealed a large IgG1, IgG2b and IgG3 fraction (FIG. 2B), which is consistent with a strong T_{helper}-2 CD4^{+} T cell response in the context of this adjuvant (Vandepapeliere et al., Vaccine, 23:2591-601, 2005). Because the humoral immune response of patients with AIH is targeted against the carboxy terminus of the SLA/LP protein (Herkel et al., Hepatology, 35:403-8, 2002), antibodies against this region were mapped with overlapping 20mer peptides. As shown in FIG. 2C, the 20mer peptide spanning amino acid residues 403-422 elicited the most vigorous antibody response in HLA-DRB1*0301-transgenic mice.

The 20mer peptide spanning amino acid residues 403-422 was significantly less vigorously recognized by nontransgenic C57BL/6 mice, which suggests HLA-DR-restricted CD4^{+} T cell help in the generation of autoantibodies against amino acid residues 403-422 of SLA/LP. After activation by HLA-DRB1*0301-restricted autoantigens, CD4^{+} T cells might, for example, provide help for B cells to produce autoantibodies, and to secrete proinflammatory cytokines and chemokines that recruit plasma cells and CD8^{+} T cells into the liver. Vice versa, autoantigen-specific B cells might bind and take up the autoantigen via specific autoantibodies on their cell surface, process it into short peptides and present these peptides on DRB1*0301 molecules to stimulate CD4^{+} T cells (Mamula, Immunol. Rev., 164:231-9, 1998).
Example 3

CELLULAR IMMUNE RESPONSE TO SLA/LP IN
HLA-DRB1*0301 TRANSGENIC MICE

To identify the SLA/LP-specific T cell epitopes presented and recognized in the context of DRB1*0301 molecules, immunized mice were sacrificed and draining lymph nodes and spleens were removed. As shown in FIG. 3A, DR expression was readily detectable on peripheral blood mononuclear cells prior to immunization of the mice. Lymph node (FIG. 3B) T cells of SLA/LP-immunized mice proliferated significantly in response to SLA/LP. Spleen-derived T cells of SLA/LP-immunized HLA-DRB1*0301-transgenic mice also proliferated (FIG. 4A) and produced IL-2 (FIG. 4B) in a dose-dependent manner in response to SLA/LP. Because of the large cell number of T cells required for epitope mapping with overlapping peptides, spleen-derived T cells of immunized mice were used for all subsequent experiments. IL-2 production was chosen as the read-out for epitope mapping experiments because of the high sensitivity of the assay.

Example 4

IDENTIFICATION OF IMMUNODOMINANT REGIONS WITHIN SLA/LP

When the immune response of SLA/LP-immunized mice was assessed with a panel of overlapping 20mer peptides spanning the entire sequence of human SLA/LP, several immunogenic regions within the SLA/LP protein were identified that were recognized by T cells (FIG. 4C). Peptides at amino acid positions 1-28, 49-76, 81-116, 137-156, 177-220, 233-284, 289-316, 329-356, 361-404, and 400-422 of SLA/LP (corresponding to residues of SEQ ID NO: 6) stimulated a significant T cell response in a sensitive ex vivo IL-2 ELISpot assay and are marked by filled bars in FIG. 4C. In contrast, the peptides marked by open bars in FIG. 4C did not generate a significant T cell response in DRB1*0301-transgenic mice.

As described in more detail below, immunogenic regions for which T cell hybrids could be established from either SLA/LP protein or peptide immunized mice are
indicated in FIG. 4D by black horizontal bars. T cell hybrids were not established for regions indicated by open horizontal bars in FIG. 4D. The inability to form T cell hybrids reactive with the regions shown by open horizontal bars in FIG. 4D likely is an experimental artefact. For example, the frequency of T cells specific for a region that stimulates only a modest T cell response may be quite low; therefore, it would be necessary to screen a relatively high number of fused T cells to find one responsive to the region of interest. It is further possible that the cellular state of T cells that respond very strongly to a particular region of SLA/LP is incompatible with the cell fusion process or continued cell culture of a fused cell. Under such circumstances, the isolation of a fused T cell line would not be expected.

The several immunogenic regions of human SLA/LP that were endogenously processed and recognized by SLA/LP-specific T cells are marked on the human SLA/LP sequence in FIG. 5. Interestingly, the immunogenic sequences located in the carboxy-terminus of SLA/LP (see also FIG. 4C) overlapped with the region that is known to be recognized by antibodies of patients with AIH (Herkel et al., Hepatology, 35:403-8, 2002).

Example 5

MAPING OF AN SLA/LP EPITOPE INVOLVED IN THE SLA/LP-SPECIFIC T CELL RESPONSE IN HLA-DR3 TRANSGENIC MICE

To further fine-map the immunodominant sequences identified as described in Example 3, mouse T cell hybrids were generated by fusing activated SLA/LP-specific T cells with the T cell receptor-negative cell line BWZ.36. Because BWZ.36 contained the beta-galactosidase reporter gene under the control of the NFAT element of the human interleukin-2 enhancer 11, the resulting hybrids expressed both the SLA/LP epitope-specific T-cell receptor and a reporter gene that can be triggered by engagement of this receptor. All isolated T cell hybrids were CD4-positive.

Results for a representative T cell hybridoma illustrate significant activation of the beta-galactosidase reporter gene by stimulation with the 20mer peptide SLA_{369-388} in
the presence of irradiated DRB1*0301-positive spleen cells (FIG. 6A, top bar). This region was interesting, because it overlapped with the SLA/LP sequence that is known to be recognized by antibodies of patients with AIH (Herkel et al., Hepatology, 35:403-8, 2002). This close proximity of B and T cell epitopes within the SLA/LP autoantigen appears to be a characteristic of autoimmune diseases (Reijonen et al., Diabetes, 53:1987-94, 2004; Galperin et al., Baillieres Clin. Gastroenterol., 10:461-81, 1996; Wucherpfennig et al., J. Clin. Invest., 100:1114-22, 1997).

As shown in FIG. 6A, T cell recognition increased further when three amino acids were removed from the amino terminus and two or three amino acids were removed from the carboxy terminus of peptide SLA369-388 (FIG. 6A, bars 2 and 3 from top). T cell recognition was significantly decreased, however, upon further stepwise truncation of the C-terminus of the peptide (FIG. 6A, bars 4-6 from top). Ultimately, peptide SLA373-386 NRLDRCLKAVRER (SEQ ID NO: 5) was identified as the optimal epitope (FIG. 6A, underlined sequence) in dose titration experiments (FIG. 6B).

To analyze MHC restriction, the same T cell hybridoma was then stimulated with the optimal peptide and DRB1*0301-positive spleen cells in the context of I-A/I-E antibody, HLA-DR antibody or isotype control antibody, respectively. As shown in FIG. 6C, T cell recognition of peptide SLA373-386 was inhibited by HLA-DR antibodies, but not by I-A/I-E antibodies and isotype controls, indicating restriction by HLA-DR and illustrating that this epitope likely would be recognized by human T cells.

Example 6

MAPPING OF ANOTHER SLA/LP EPITOPE INVOLVED IN THE SLA/LP-SPECIFIC T CELL RESPONSE IN HLA-DR3 TRANSGENIC MICE

In a manner similar to that described in Example 5, another DRB1*0301-restricted optimal epitope was mapped in SLA/LP and, in particular, within sequence SLA184-204 (see FIG. 7). Stepwise amino- and carboxy-terminal truncation identified SLA186-197 LIQQGARVGRID (SEQ ID NO: 7) as an optimal minimum epitope (FIG. 7A, underlined sequence) in this region of the protein; however,
several related epitopes had nearly the same activity. Deletion of the carboxy-terminal aspartic acid of peptide SLA<sub>186-197</sub> reduced recognition, as did deletion of the amino-terminal lysine and carboxy-terminal aspartic acid of peptide SLA<sub>186-197</sub> (FIG. 7B). Antibody blocking experiments revealed that the SLA<sub>186-197</sub> epitope was also restricted by HLA-DR (FIG. 7C) indicating this peptide likely would be recognized by human T cells.

Example 7

**MAPPING OF SLA/LP EPITOPES IN THE SLA/LP N-TERMINUS**

In a manner similar to that described in Example 5, an optimal epitope was mapped within the first twenty amino acids of SLA/LP (i.e., SLA<sub>1-20</sub>). An optimal epitope was identified as SLA<sub>7-18</sub> CFWRRIHGIGR (SEQ ID NO: 1).

There are two reported SLA/LP sequences (GenBank Accession Nos. AAD33963 and AJ277541) (Wies et al., Lancet, 355:1510-5, 2000). One sequence (JD.1, GenBank Accession No. AAD33963) is derived from an SLA/LP-encoding clone isolated from Jurkat-cell cDNA library. The other sequence (L-D.1, GenBank Accession No. AJ277541) is derived from an SLA/LP-encoding clone isolated from a human liver cDNA library. The L-D.1 sequence includes a 156 base pair insert between bases 111 and 112 of the JD.1 sequence (Weis et al., Lancet, 355:1510-1515, 2000).

SLA/LP-specific T cells were isolated from mice immunized with the Jurkat cell SLA/LP protein sequence. Due to the difference in sequence between Jurkat cell and human liver SLA/LP in the N-terminal region, CFWRRIHGIGR-reactive T cells may not recognize a corresponding liver-derived SLA/LP sequence, which includes 52 additional amino acids between residues 5 and 6 of the SLA<sub>7-18</sub> CFWRRIHGIGR sequence.

Nevertheless, it is expected that T cells will recognize the corresponding epitopes from the human liver SLA/LP sequence (e.g., CFWRGKCPENG and RRHYRFIHGIGR; SEQ ID NOs: 12 and 13, respectively). Moreover, it is expected
that T cell epitopes exist in the 52 amino acids particular to human liver SLA/LP
(GKCPENGWDESTLELFLHELAIMDSNNFLGNCVGERGRVASALVARRHYR; SEQ ID NO: 14) and/or that T cells will recognize the amino acids common to the
Jurkat cell and human liver SLA/LP sequences, which, in the human liver SLA/LP
sequence, flank the insertion points of the additional 53 amino acids (e.g., CFWRR
(residues 7-11 of SEQ ID NO: 6) and GIGR).

In summary, Examples 5-7 illustrate the use of a humanized,
HLA-DRB1*0301-transgenic mouse model for immunization with human SLA/LP and
the identification of at least two HLA-DR-restricted SLA/LP epitopes that are
recognized by CD4+ T cells. Interestingly, one of these epitopes (FIG. 5) overlapped the
sequence of the major B cell SLA/LP autoantigen, which is recognized by patients with
anti-SLA/LP positive AIH (Herkel et al., Hepatology, 35:403-8, 2002; Wies et al.,
Lancet, 355:1510-5, 2000). HLA-DR-restricted SLA/LP epitopes that are recognized by
CD4+ T cells can be useful for diagnosis and treatment of human AIH.

Example 8

GENERATION OF DRB1*0301/SLA184-198 AND DRB1*0301/SLA184-198
TETRAMERS TO DETECT SLA/LP-SPECIFIC CD4+ T CELLS IN VIVO

This Example demonstrates that the identified HLA-DR-restricted epitopes can
be used to detect SLA/LP-specific CD4+ T cells in vivo.

Fluorescently labeled tetrameric DRB1*0301 complexes that present the
SLA/LP epitopes of interest to DRB1*0301-restricted CD4+ T cells were generated.
DRB1*0301 tetramers were originally synthesized for epitope SLA184-198 and epitope
SLA186-197. The SLA186-197-containing tetramer did not optimally fold with epitope
SLA186-197; however, a slightly longer peptide (i.e., SLA184-198; MHLIQGARVGRID
(SEQ ID NO: 11)), which included the SLA186-197 epitope, performed as desired. This
finding indicates that the addition of one or a few amino acid residues to a disclosed
epitope can facilitate the assembly of an epitope-containing peptide with MHC
molecules to form a multimer useful for detecting T cells.
The expected folding of SLA/LP antigen-containing tetramers can be determined in a variety of ways, including functional and/or structural analyses. A representative functional assay can measure, for example, peptide binding to MHC complexes. For structural analyses, circular dichroism, infrared spectroscopy, or nuclear magnetic resonance spectroscopy could be used to analyze secondary structure content, e.g., percentage of the polypeptide chain in alpha helix or beta sheet conformation. Alternatively, one could assay for correct assembly of the tetramer by identifying complexes of the predicted size, for example using analytical ultracentrifugation, native acrylamide gels, or size exclusion chromatography.

Both, the DRB1*0301/SLA_{373-386} tetramer and the DRB1*0301/SLA_{184-198} tetramer were first titered and validated with CD4^+ T cells of immunized HLA-DRB1*0301-transgenic mice. As shown in FIG. 8A, a large DRB1*0301/SLA_{373-386} tetramer-positive CD4^+ T cell population was identified in the spleens of two representative DRB1*0301-transgenic, immunized mice, but not in a DRB1*0301-transgenic, nonimmunized control mouse. A DRB1*0301/SLA_{184-198} tetramer-positive CD4^+ T cell population of comparable size was detected in the spleen two additional, immunized mice (FIG. 8B), but not in a nonimmunized control mouse. Both DRB1*0301-tetramer-positive CD4^+ T cell populations expressed CD11a and, in comparison to DRB1*0301/epitope-tetramer-negative CD4^+ T cells, had down-regulated CD62L expression indicating that they had lost their lymph node homing potential as expected for antigen-induced effector T cells. Serum alanine aminotransferase (ALT) levels remained within the upper limit of normal in all immunized mice and no significant lymphocyte infiltrate was detected in the liver.

The finding that none of the SLA/LP-immunized HLA-DRB1*0301-transgenic mice developed serological or histological evidence of autoimmune hepatitis is not unexpected and can be explained by several factors. Sequence differences between mouse and human SLA/LP, which affect five amino acids in epitope SLA_{373-386}, may be one of the contributing factors. In addition, recent studies in the autoimmune diabetes model suggest that the frequency of autoantigen-specific T cells needs to be quite high

In contrast to other organs, the microenvironment of the liver has been reported to favor immune tolerance rather than inflammation (Crispe, Nat. Rev. Immunol., 3:51-62, 2003) and it may therefore be more difficult to induce autoimmune disease in the liver (Wirth et al., J. Immunol., 154:2504-15, 1995; Shimizu et al., J. Immunol., 161:4520-9, 1998). Additional factors, such as tissue destruction by unrelated mechanisms (toxic or infectious) might be necessary to induce cytokine release, activation of antigen-presenting cells (von Herrath et al., Immunity, 3:727-38, 1995), upregulation of MHC class II and costimulatory molecules, and to mediate autoimmune hepatitis. Since SLA/LP (Wies et al., Lancet, 355:1510-5, 2000) and LKM, the second cloned autoantigen in AIH, are cytosolic enzymes (Manns et al., J. Clin. Invest., 83:1066-72, 1989), it is also possible that specific enzyme-substrate interactions initiate hepatocyte injury and death (Wang et al., Hepatology, 42:400-10, 2005), which is then followed by transport of cell-associated autoantigens into the regional lymph nodes and priming of autoantigen-specific T and B cells. Whatever the factors are that initiate the autoimmune process, the inflammatory reaction appears to be mediated and continuously fueled by autoantigen-specific T cells once chronic autoimmune hepatitis is established.

This Example demonstrates, at least, that DRB1*0301/SLA\textsubscript{373-386} and DRB1*0301/SLA\textsubscript{184-198} presenting molecules (e.g., tetramers) can successfully be used to specifically detect SLA/LP-specific T cells in vivo.
Example 9

**DRB1*0301/SLA373-386 TETRAMER DETECTS AUTOACTIVE, SLA373-386-SPECIFIC T CELL POPULATION IN HUMAN AIH PATIENT**

This Example demonstrates that DRB1*0301-restricted SLA373-386 peptide specifically stimulated PBMCs from a patient with anti-SLA/LP-positive AIH, but did not stimulate PBMCs from a control patient, who had recovered from hepatitis B virus infection but did not suffer from AIH. Both patients were HLA-DRB1*0301 positive. PBMC were labelled with CFSE and stimulated with the DRB1*0301-restricted SLA373-386 peptide and, as negative control, with the well characterized DRB1*0301-restricted peptide HCV261-269 (Diepold et al., *J. Virol.*, 71:6011-6019, 1997). When PBMC of the anti-SLA/LP-positive AIH patient were stimulated with the SLA373-386 peptide, 0.17% of all CD8*CD3* cells stained with the DRB1*0301/SLA373-386 tetramer (FIG. 9A, left panel). When the unrelated, DRB1*0301-restricted HCV peptide was used for stimulation, only 0.02% CD8*CD3* cells stained with the DRB1*0301/SLA373-386 tetramer (FIG. 9B, right panel), indicating that the autoreactive, SLA373-386-specific T cell population responded in peptide-specific manner. PBMC of the DRB1*0301-positive control patient without AIH tested negative (FIG. 9B).

To analyze the specificity of the tetramer, tetramer-positive and tetramer-negative cells of the anti-SLA/LP-positive AIH patient were then compared with regards to activation and proliferation. As shown in the left panel in FIG. 9A, a greater percentage of tetramer-positive cells than tetramer-negative cells had proliferated on day 10 of stimulation with the SLA373-386 peptide (47.0% versus 6.2%). Proliferation correlated with the expression of activation markers on tetramer-positive cells. In contrast, few cells (3.7%) had proliferated when the culture was stimulated with the unrelated, DRB1*0301-restricted HCV control peptide (FIG. 9A, right panel). Thus, the DRB1*0301/SLA373-386 tetramer was successfully used to specifically detect SLA/LP-specific T cells in the blood of a patient with anti-SLA/LP-positive AIH.
This Example demonstrates that the identified T cell epitopes can now be used to detect SLA/LP-specific T cells \textit{ex vivo}. DRB1*0301-tetramers that present the respective epitope stain autoreactive T cells at the single cell level. This finding further enables, among other things, the monitoring of AIH-specific T cells during progression of disease and/or immunosuppressive therapy.

In view of the many possible embodiments to which the principles of the present disclosure may be applied, it should be recognized that the illustrated embodiment is only a preferred example of the disclosure and should not be taken as a limitation on the scope of the disclosure. Rather, the scope of the disclosure is defined by the following claims. We therefore claim as our disclosure all that comes within the scope and spirit of these claims.
- 60 -

CLAIMS

1. An isolated immunogenic region peptide comprising an amino acid sequence selected from the group consisting of:
   (a) SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10, or 11;
   (b) sequences having at least 90% sequence identity to any of the sequences in (a); and
   (c) highly conserved variants of any of the sequences in (a);
   wherein the peptide comprises an immunogenic region recognizable by T cells,
   and wherein the peptide is 50 or fewer residues in length.

2. The isolated immunogenic region peptide of claim 1, wherein the peptide is 30 or fewer residues in length.

3. The isolated immunogenic region peptide of claim 2, wherein the peptide is 25 or fewer residues in length.

4. The isolated immunogenic region peptide of claim 3, wherein the peptide is 20 or fewer residues in length.

5. The isolated immunogenic region peptide of claim 1, wherein the peptide consists of SEQ ID NO: 1, 2, 3, 4, 5, 7, 9, 10, or 11.

6. An altered peptide ligand derived from the isolated immunogenic region peptide of claim 5, wherein the altered peptide ligand comprises at least one amino acid substitution, and wherein the altered peptide ligand comprises an immunogenic region recognizable by T cells.
7. The altered peptide ligand of claim 6, wherein the altered peptide ligand is 30 or fewer residues in length.

8. The altered peptide ligand of claim 7, wherein the altered peptide ligand is 25 or fewer residues in length.

9. The altered peptide ligand of claim 8, wherein the altered peptide ligand is 20 or fewer residues in length.

10. The altered peptide ligand of claim 6, wherein the amino acid substitutions comprise conservative substitutions.

11. The altered peptide ligand of claim 6, wherein the amino acid substitutions comprise non-conservative substitutions.

12. An antagonist derived from the isolated immunogenic region peptide of claim 5, wherein the antagonist comprises an immunogenic region recognizable by T cells, and wherein the antagonist antagonizes an activity of the isolated immunogenic region peptide.

13. The antagonist of claim 12, wherein the antagonist is a peptide antagonist.

14. The antagonist of claim 12, wherein the antagonist is a non-peptide antagonist.
15. A method of diagnosing or monitoring an autoimmune disease comprising:
   obtaining a sample from a subject; and
   quantifying T cells in the sample that are reactive with the isolated immunogenic
   region peptide of claim 1.

16. The method of claim 15, wherein the sample is blood, lymphatic fluid,
   lymphoid tissue, spleen tissue, or liver tissue.

17. The method of claim 15, wherein the method further comprises
determining the function of T cells in the sample that are reactive with the isolated
immunogenic region peptide of claim 1.

18. The method of claim 17, wherein the determining the function of T cells
in the sample comprises detecting or measuring secreted or intracellular cytokines,
detecting or measuring proliferation, detecting or measuring cytotoxicity, or detecting or
measuring functional interaction with B cells, CD8 cells or dendritic cells.

19. The method of claim 15, wherein the method further comprises
comparing with a baseline value the quantity of T cells in the sample that are reactive
with the isolated immunogenic region peptide of claim 1.

20. The method of claim 19, wherein the baseline value is the quantity of
T cells in a sample taken from the same subject at an earlier time that are reactive with
the isolated immunogenic region peptide of claim 1.

21. The method of claim 19, wherein the baseline value is the average
quantity of T cells in samples taken from a population of subjects with autoimmune
hepatitis that are reactive with the isolated immunogenic region peptide of claim 1.
22. The method of claim 19, wherein the baseline value is the average quantity of T cells in samples taken from a population of subjects without autoimmune hepatitis that are reactive with the isolated immunogenic region peptide of claim 1.

23. The method of claim 15, wherein quantifying T cells in the sample that are reactive with the isolated immunogenic region peptide of claim 1 comprises using an ELISpot assay.

24. The method of claim 15, wherein quantifying T cells in the sample that are reactive with the isolated immunogenic region peptide of claim 1 comprises using a multimeric MHC/peptide antigen binding complex, wherein the peptide antigen comprises the isolated immunogenic region peptide of claim 1.

25. A kit for diagnosing or monitoring autoimmune hepatitis in a subject, wherein the kit comprises the isolated immunogenic region peptide of claim 1, a container, and instructions for using the kit.

26. A method of treating autoimmune hepatitis in a subject comprising:

administering to the subject a therapeutically effective amount of the altered peptide ligand of claim 6, thereby treating the autoimmune hepatitis.

27. The method of claim 26, wherein administering the isolated immunogenic region comprises parenteral administration.

28. The method of claim 27, wherein parenteral administration comprises subcutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery.
29. The method of claim 26, wherein administering the altered peptide ligand comprises repeated administration.

30. The method of claim 29, wherein administration is repeated daily, weekly, or monthly for at least three administrations.

31. A method of treating autoimmune hepatitis in a subject comprising: administering to the subject a therapeutically effective amount of the antagonist of claim 12, thereby treating the autoimmune hepatitis.

32. The method of claim 31, wherein administering the antagonist comprises parenteral administration.

33. The method of claim 32, wherein parenteral administration comprises subcutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery.

34. The method of claim 31, wherein administering the antagonist comprises repeated administration.

35. The method of claim 34, wherein administration is repeated daily, weekly, or monthly for at least three administrations.

36. A method of treating autoimmune hepatitis in a subject comprising: administering to the subject a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex, wherein the peptide antigen comprises the isolated immunogenic region peptide of claim 1, and wherein the multimeric MHC/peptide antigen complex is conjugated to a toxin moiety, thereby treating the autoimmune hepatitis in the subject.
37. The method of claim 36, wherein administering the antagonist comprises parenteral administration.

38. The method of claim 37, wherein parenteral administration comprises sub-cutaneous administration, intramuscular administration, intravenous administration, intra-arterial administration, or infusion into the hepatic artery.

39. The method of claim 36, wherein administering the antagonist comprises repeated administration.

40. The method of claim 39, wherein administration is repeated daily, weekly, or monthly for at least three administrations.

41. The method of claim 36, wherein the toxin moiety is ricin, abrin, diphtheria toxin, a maytansinoid, or cisplatin.

42. The method of claim 36, wherein the multimeric MHC/peptide antigen complex is a dimeric, trimeric, tetrameric, or pentameric complex.

43. A method of treating autoimmune hepatitis in a subject comprising:
   isolating immune cells from the subject;
   ablating T cells reactive with the isolated immunogenic region peptide of claim 1 with a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex; and
re-introducing the immune cells to the subject, wherein the peptide antigen comprises the isolated immunogenic region peptide of claim 1, and wherein the multimeric MHC/peptide antigen complex is conjugated to a toxin moiety, thereby treating the autoimmune hepatitis in the subject.

44. The method of claim 43, wherein the toxin moiety is ricin, abrin, diphtheria toxin, a maytansinoid, or cisplatin.

45. The method of claim 43, wherein the multimeric MHC/peptide antigen complex is a dimeric, trimeric, tetrameric, or pentameric complex.

46. A method of treating autoimmune hepatitis in a subject comprising: isolating immune cells from the subject; depleting T cells reactive with the isolated immunogenic region peptide of claim 1 with a therapeutically effective amount of a multimeric MHC/peptide antigen binding complex; and re-introducing the immune cells to the subject, wherein the peptide antigen comprises the isolated immunogenic region peptide of claim 1, and wherein the multimeric MHC/peptide antigen complex is conjugated to a separation moiety.

47. The method of claim 46, wherein the separation moiety is phycoerythrin.

48. The method of claim 46, wherein the multimeric MHC/peptide antigen complex is a dimeric, trimeric, tetrameric, or pentameric complex.
FIG. 1

Immunization with human SLA/LP

→ Analysis of SLA/LP-specific autoantibody response

1. Detection of SLA/LP-specific T cells

2. Generation of T cell hybridoma
   Fusion of T cells with TCR-negative lymphoma line
   that expresses the β-gal upon stimulation of IL-2 promoter

3. Mapping of T cell fine-specificity
   with overlapping SLA-peptides (20mers)

4. Identification of optimal
   peptide sequences (epitopes)

5. Identification of HLA-molecule
   presenting the epitopes

6. Generation and verification of
   HLA-DRB1*0301 tetramer
   presenting the epitope of interest

7. Identification of tetramer-positive,
   SLA/LP-specific T cells
FIG. 3

A

[Graph showing cell number distribution with HLA-DR-FITC on the x-axis and cell number on the y-axis.]

B

[Bar graph showing stimulation index with PMA + Ionomycin and SLA as categories.]
FIG. 5

MSTSYGCFWRFHIGRSGDISAVQPKAAGSSLNKITNSLVDIIKLA 50
GVHTVANCVFVPMATGMSLTLCLFLTRHHRPKAYIIPRIDQQSCFKSM 100
ITAGFEPVVIENVLECGDELRTDKAVEAKVQELGPDCILCIHSTSTSCFAP 150
RPDRLEELAVICANYDIPHIVNNAYGVQSSKCMHLLIQGERVGRDADFV 200
QSDLKFMVPVGAIIFQGFDNSFIEISKMYPGRASAPSLDVLITLLSL 250
GSNGYKLLKERRKEMFSYLSNQIKLSEAYNERLLHTPHPNPIASMPLTK 300
LDEHRDKAVTQLGSMQLFTQVSGARVVPILGSMQTVSGYTFGPMHMHTNNY 350
PCAYLNAASAGMKMOVQVDFINRLDRCRKLAVRKRKESDDNYDKTEDV 400
DIEEMLKLIDNFLNLLDTYQDASS 422
FIG. 9

A 10-Day Stimulation with SLA\textsubscript{273-283} Peptide

B 10-Day Stimulation with HCV\textsubscript{271-280} peptide

[Graphs showing cellular proliferation with different cell markers and CFSE]
SEQUENCE LISTING

The Government of the United States of America, as represented by the Secretary of the Dept. of Health and Human Services
Rehmann, Barbara
Christina, Weiler-Normann
Mix, Heiko
Lohse, Ansgar W.

IMMUNOGENIC T CELL TARGETS IN AUTOIMMUNE HEPATITIS AND METHODS OF USE

4239-67825-02
US 60/659,513
2005-03-07
14
PatentIn version 3.3

1
12
PRT
Homo sapien

Cys Phe Trp Arg Arg Phe Ile His Gly Ile Gly Arg
1 5

2
13
PRT
Homo sapien

2

Ile Ile Trp Pro Arg Ile Asp Gln Lys Ser Cys Phe Lys
1 5

3
11
PRT
Homo sapien

3

Ile Gln Gln Gly Ala Arg Val Gly Arg Ile Asp
1 5

4
17
PRT
Homo sapien

4

Glu Glu Met Ala Leu Lys Leu Asp Asn Val Leu Leu Asp Thr Tyr Gln
1 5 10 15

Page 1
Asp

<formatted_text>5</formatted_text>
<formatted_text>14</formatted_text>
<formatted_text>PRT</formatted_text>
<formatted_text>Homo sapien</formatted_text>
<formatted_text>5</formatted_text>

Asn Arg Leu Asp Arg Cys Leu Lys Ala Val Arg Lys Glu Arg

<formatted_text>5</formatted_text>
<formatted_text>10</formatted_text>

<formatted_text>6</formatted_text>
<formatted_text>422</formatted_text>
<formatted_text>PRT</formatted_text>
<formatted_text>Homo sapien</formatted_text>
<formatted_text>6</formatted_text>

Met Ser Thr Ser Tyr Gly Cys Phe Trp Arg Arg Phe Ile His Gly Ile

<formatted_text>1</formatted_text>
<formatted_text>5</formatted_text>
<formatted_text>10</formatted_text>
<formatted_text>15</formatted_text>

Gly Arg Ser Gly Asp Ile Ser Ala Val Gln Pro Lys Ala Ala Gly Ser

<formatted_text>20</formatted_text>
<formatted_text>25</formatted_text>
<formatted_text>30</formatted_text>

Ser Leu Leu Asn Lys Ile Thr Asn Ser Leu Val Leu Asp Ile Ile Lys

<formatted_text>35</formatted_text>
<formatted_text>40</formatted_text>
<formatted_text>45</formatted_text>

Leu Ala Gly Val His Thr Val Ala Asn Cys Phe Val Val Pro Met Ala

<formatted_text>50</formatted_text>
<formatted_text>55</formatted_text>
<formatted_text>60</formatted_text>

Thr Gly Met Ser Leu Thr Leu Cys Phe Leu Thr Leu Arg His Lys Arg

<formatted_text>65</formatted_text>
<formatted_text>70</formatted_text>
<formatted_text>75</formatted_text>
<formatted_text>80</formatted_text>

Pro Lys Ala Lys Tyr Ile Ile Trp Pro Arg Ile Asp Gln Lys Ser Cys

<formatted_text>85</formatted_text>
<formatted_text>90</formatted_text>
<formatted_text>95</formatted_text>

Phe Lys Ser Met Ile Thr Ala Gly Phe Glu Pro Val Val Ile Glu Asn

<formatted_text>100</formatted_text>
<formatted_text>105</formatted_text>
<formatted_text>110</formatted_text>

Val Leu Glu Gly Asp Glu Leu Arg Thr Asp Leu Lys Ala Val Glu Ala

<formatted_text>115</formatted_text>
<formatted_text>120</formatted_text>
<formatted_text>125</formatted_text>

Lys Val Gln Glu Leu Gly Pro Asp Cys Ile Leu Cys Ile His Ser Thr

<formatted_text>130</formatted_text>
<formatted_text>135</formatted_text>
<formatted_text>140</formatted_text>

Thr Ser Cys Phe Ala Pro Arg Val Pro Asp Arg Leu Glu Glu Leu Ala

<formatted_text>145</formatted_text>
<formatted_text>150</formatted_text>
<formatted_text>155</formatted_text>
<formatted_text>160</formatted_text>

Val Ile Cys Ala Asn Tyr Asp Ile Pro His Ile Val Asn Asn Ala Tyr

<formatted_text>165</formatted_text>
<formatted_text>170</formatted_text>
<formatted_text>175</formatted_text>
Gly Val Gln Ser Ser Lys Cys Met His Leu Ile Gln Gln Gly Ala Arg
180 185 190

Val Gly Arg Ile Asp Ala Phe Val Gln Ser Leu Asp Lys Asn Phe Met
195 200 205

Val Pro Val Gly Gly Ala Ile Ile Ala Gly Phe Asn Asp Ser Phe Ile
210 215 220

Gln Glu Ile Ser Lys Met Tyr Pro Gly Arg Ala Ser Ala Ser Pro Ser
225 230 235 240 245

Leu Asp Val Leu Ile Thr Leu Leu Ser Leu Gly Ser Asn Gly Tyr Lys
245 250 255

Lys Leu Leu Lys Glu Arg Lys Glu Met Phe Ser Tyr Leu Ser Asn Gln
260 265 270

Ile Lys Lys Leu Ser Glu Ala Tyr Asn Glu Arg Leu Leu His Thr Pro
275 280 285

His Asn Pro Ile Ser Leu Ala Met Thr Leu Lys Thr Leu Asp Glu His
290 295 300 305

Arg Asp Lys Ala Val Thr Gln Leu Gly Ser Met Leu Phe Thr Lys Gln
310 315 320 325

Val Ser Gly Ala Arg Val Val Leu Gly Ser Met Gln Thr Val Ser
330 335

Gly Tyr Thr Phe Arg Gly Phe Met Ser His Thr Asn Asn Tyr Pro Cys
340 345 350

Ala Tyr Leu Asn Ala Ala Ser Ala Ile Gly Met Lys Met Gln Asp Val
355 360 365

Asp Leu Phe Ile Asn Arg Leu Asp Arg Cys Leu Lys Ala Val Arg Lys
370 375 380

Glu Arg Ser Lys Glu Ser Asp Asp Asn Tyr Asp Lys Thr Glu Asp Val
385 390 395 400 405

Asp Ile Glu Glu Met Ala Leu Lys Leu Asp Asn Val Leu Leu Asp Thr
410 415

Tyr Gln Asp Ala Ser Ser
420

Page 3
Leu Ile Gln Gln Gly Ala Arg Val Gly Arg Ile Asp
1 5 10

Val Leu Val Leu Asn Pro Ser Val Ala
1 5

Ile Gln Gln Gly Ala Arg Val Gly Arg Ile
1 5 10

Arg Leu Asp Arg Cys Leu Lys Ala Val Arg Lys Glu
1 5 10

Met His Leu Ile Gln Gln Gly Ala Arg Val Gly Arg Ile Asp
1 5 10

Cys Phe Trp Arg Arg Gly Lys Cys Pro Glu Asn Gly
1 5 10
<210> 13
<211> 12
<212> PRT
<213> Homo sapien

<table>
<thead>
<tr>
<th>Arg</th>
<th>Arg</th>
<th>His</th>
<th>Tyr</th>
<th>Arg</th>
<th>Phe</th>
<th>Ile</th>
<th>His</th>
<th>Gly</th>
<th>Ile</th>
<th>Gly</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<210> 14
<211> 52
<212> PRT
<213> Homo sapien

<table>
<thead>
<tr>
<th>Gly</th>
<th>Lys</th>
<th>Cys</th>
<th>Pro</th>
<th>Glu</th>
<th>Asn</th>
<th>Gly</th>
<th>Trp</th>
<th>Asp</th>
<th>Glu</th>
<th>Ser</th>
<th>Thr</th>
<th>Leu</th>
<th>Glu</th>
<th>Leu</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leu</th>
<th>His</th>
<th>Glu</th>
<th>Leu</th>
<th>Ala</th>
<th>Ile</th>
<th>Met</th>
<th>Asp</th>
<th>Ser</th>
<th>Asn</th>
<th>Asn</th>
<th>Phe</th>
<th>Leu</th>
<th>Gly</th>
<th>Asn</th>
<th>Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>25</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gly</th>
<th>Val</th>
<th>Gly</th>
<th>Val</th>
<th>Arg</th>
<th>Gly</th>
<th>Val</th>
<th>Arg</th>
<th>Val</th>
<th>Ala</th>
<th>Ser</th>
<th>Ala</th>
<th>Leu</th>
<th>Val</th>
<th>Ala</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>40</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arg</th>
<th>His</th>
<th>Tyr</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>