[0059] ~~The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."~~

[0060] As used herein and in the claims, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several classes of structurally related proteins that function as part of the immune response of an animal or recipient, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins.

[0061] As used herein the terms "immunogenic agent" or "immunogen" or "antigen" are used interchangeably to describe a molecule capable of inducing an immunological response against itself on administration to a recipient, either alone, in conjunction with an adjuvant, or presented on a display vehicle.

[0062] As used herein, an "amino molecule" refers to any amino acid, amino acid derivative, or amino acid mimic known in the art. In certain embodiments, the residues of the proteinaceous molecule are sequential, without any non-amino molecule interrupting the sequence of amino molecule residues. In other embodiments, the sequence may comprise one or more non-amino molecule moieties. In particular embodiments, the sequence of residues of the proteinaceous molecule may be interrupted by one or more non-amino molecule moieties.

[0063] Accordingly, the term "proteinaceous composition" encompasses amino molecule sequences comprising at least one of the 20 common amino acids in naturally synthesized proteins, or at least one modified or unusual amino acid.

[0064] As used herein, the term "treatment" or "treating" means any treatment of a disease or condition in a patient, including:

- preventing or protecting against the disease or condition, that is, causing the clinical symptoms not to develop, for example, in a subject at risk of suffering from such a disease or condition, thereby substantially averting onset of the disease or condition;
- inhibiting the disease or condition, that is, arresting or suppressing the development of clinical symptoms; and/or

- relieving the disease or condition that is, causing the regression of clinical symptoms.

[0065] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the ~~spirit and~~ scope of the invention will become apparent to those skilled in the art from this detailed description.

[0066] Observations to date (Han et al., 2005) suggested that, to be effective in autoimmunity, peptide therapy would have to target multiple epitope specificities. Soluble peptides can induce peptide-specific T-cell tolerance, but cannot blunt poly-specific autoimmune responses. The inventors reasoned that it would be highly impractical to accomplish this with peptides because, in the case of IGRP alone, this would require several milligrams of peptides per dose. Because peptides are much more tolerogenic (i.e., at lower amounts) when bound to MHC molecules on fixed APCs (Miller et al., 1979), it was contemplated that systemic delivery of antigen/MHC complexes, e.g., peptide/MHC complexes (without costimulatory molecules) on nanospheres might be more tolerogenic than peptides alone. This thought evolved from the availability of a reagent initially conceived to image islet inflammation. The inventors sought to specifically deliver a probe amenable to magnetic resonance (MR) imaging to circulating 8.3-like $CD8^+$ T-cells (iron oxide nanospheres coated with NRP-V7/K^d complexes) (Moore et al., 2004). In particular, the inventors contemplated coating these nanospheres with several different antigen/MHC complexes as a way to induce the simultaneous deletion of multiple T-cell specificities below the threshold required for T1D development. Most surprisingly, it was found that pMHC complexes attached to a solid support function by expanding, in an epitope-specific manner, autoantigen-experienced autoreactive CD8+ cells that suppress the recruitment of other autoantigenic specificitiesThis therapeutic avenue exploits a new paradigm in the progression of chronic autoimmune responses that enables the rational design of disease-specific 'nanovaccines' capable of blunting autoimmunity without impairing systemic immunity, a long sought-after goal in the therapy of these disorders. According to the above paradigm, any *single* epitope (pMHC) specificity involved in the disease (among hundreds) can be used, when coated as a ligand on NPs, to blunt complex autoimmune responses. The particle/solid support

accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, ischemic re-perfusion disorder, reperfusion injury of myocardial or other tissues, lymphomatous tracheobronchitis, inflammatory dermatoses, dermatoses with acute inflammatory components, multiple organ failure, bullous diseases, renal cortical necrosis, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, narcolepsy, acute serious inflammation, chronic intractable inflammation, pyelitis, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

VI. EXAMPLES

[0154] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. T he present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the ~~spirit~~ scope of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

T1D Protection by Treatment with Peptide/MHC-Coated Nanospheres

[0155] Diabetes protection by treatment with super-paramagnetic nanospheres coated with NRP-V7/K^d monomers. To study whether NRP-V7/K^d-coated nanospheres are tolerogenic in vivo, 8.3-TCR-transgenic NOD mice will be treated (also referred to as 8.3-NOD or Vα17.4+TCR-TG mice further below) with several i.v. injections of a small volume of nanospheres (5 µl, carrying 0.6 µg of NRP-V7, once every 3 days). It is contemplated that the transgenic high-avidity $IGRP_{206-214}$-reactive splenic CD8+ T-cell pools of these mice will be significantly depleted. It is also contemplated that the non deleted $CD8^+$T cells will be anergized by the treatment.

Claims

1. A composition of high particle density nanoparticles comprising ~~from 10 to 1000~~ antigen-MHC complexes covalently coupled to ~~a~~ biocompatible bioabsorbable nanospheres for use in a method for diagnosing, preventing or treating an autoimmune disorder when administered to a subject, wherein the composition of high density nanoparticles comprises higher numbers of smaller nanoparticles ~~in an amount sufficient~~ to expand anti-pathogenic autoreactive T cells.

2. A composition of high particle density nanoparticles comprising ~~from 10 to 1000~~ antigen-MHC complexes covalently coupled to ~~a~~ biocompatible bioabsorbable nanospheres for use in a method for expanding and/or developing populations of anti-pathogenic autoreactive T-cells in a subject, ~~when administered in an amount and frequency sufficient~~wherein the composition of high particle density nanoparticles comprises higher numbers of smaller nanoparticles to expand said populations.

3. A composition of high particle density nanoparticles comprising antigen-MHC complexes covalently coupled to biocompatible nanospheres for use in a method for inhibiting the onset of an auto-immune disease when administered to a subject, wherein the MHC of the antigen-MHC complexes comprise MHC class II molecules, and wherein the high particle density composition comprises higher numbers of smaller nanoparticles to expand anti-pathogenic autoreactive T cells.

4. A composition of high particle density nanoparticles comprising antigen-MHC complexes covalently coupled to biocompatible nanospheres for use in a method for treating the inflammatory component of an autoimmune disease when administered to a subject suffering from an autoimmune disease having an inflammatory component and said antigen is specific for the autoimmune disease, wherein the high particle density composition comprises higher numbers of smaller nanoparticles to expand anti-pathogenic autoreactive T cells which express IL-10 permitting said TR1 cells to accumulate at the site of said autoimmune disease whereby IL-10 is allowed to accumulate at said site under conditions which result in a reduction of the inflammatory component of the autoimmune disease.

~~3~~5. The composition for the use of any one of Claims 1 to 4 ~~or Claim 2~~, wherein the antigens of the antigen-MHC complexes comprise a plurality of auto-antigen epitopes ~~are contained in said auto-antigen epitope-MHC biocompatible bioabsorbable nanosphere complexes~~.

~~4~~6. The composition for the use of Claim ~~3~~5, wherein said plurality of auto-antigen epitopes are derived from a single auto-antigen or a plurality of auto-antigens.

~~5~~7. The composition for the use of any one of Claims ~~4~~1 to 6, wherein the expanded population of anti-pathogenic autoreactive T-cells are antigen specific but suppress in a non-antigen-specific manner.

~~6~~8. The composition for the use of any one of Claims ~~2, 4 or 5~~1 to 7, wherein the administration of said biocompatible bioabsorbable nanosphere complex depletes the population of cognate or non-cognate pathogenic autoreactive T cells.

~~7~~9. The composition for the use of any one of Claims 1 to 7, ~~An auto-antigen epitope-MHC-biocompatible bioabsorbable~~ wherein the nanosphere~~s complex comprising~~ comprise ~~a~~ biocompatible core~~s~~ and a biodegradable bioabsorbable coating on the outer surface of said core~~s and from 10 to 1000 antigen-MHC complexes covalently coupled to a biocompatible bioabsorbable nanosphere~~.

~~8~~10. The ~~auto-antigen epitope-MHC biocompatible bioabsorbable nanosphere complex~~composition for the use of Claim ~~7~~9, wherein said biocompatible core~~s is~~ are ~~composed of~~ selected from iron, iron (III) oxide, a polymer, and gold.

~~9~~11. The ~~auto-antigen epitope-MHC biocompatible bioabsorbable nanosphere complex~~composition for the use of Claim ~~7~~9, wherein said biodegradeble bioabsorbable coating is selected from dextran, mannitol, and poly(ethylene glycol).

~~10~~12. The ~~auto-antigen epitope-MHC biocompatible bioabsorbable nanosphere complex~~composition for the use any one of Claims ~~7~~1 to 11, wherein said covalent coupling is via a linker.

~~11~~13. ~~A~~ The composition ~~comprising from 10 to 1000 antigen-MHC complexes covalently coupled to a biocompatible nanosphere for use in a method for inhibiting the onset of an auto-immune disease when administered to a subject in an amount sufficient to expand anti-pathogenic autoreactive T cells wherein the MHC molecule comprises MHC class II molecules~~for the use of any one of Claims 1 to 12, wherein said autoreactive T-cells are CD4+ TR1 cells.

~~12~~14. The composition for the use of Claim ~~11~~13, wherein said ~~autoreactive T cells are CD4+ TR1 cells and optionally wherein the~~ CD4+ TR1 cells are characterized by IL-10 and IFNγ expression.

~~13~~15. ~~A~~ The composition ~~comprising from 10 to 1000 antigen-MHC complexes covalently coupled to a biocompatible nanosphere for use in a method for treating the inflammatory component of an autoimmune disease when administered to a subject suffering from an autoimmune disease having~~

~~an inflammatory component an antigen-MHC complex operatively coupled to a biocompatible nanosphere~~for the use of any one of Claims 1 to 2 and 4 to 14, wherein the MHC ~~molecule comprises~~of the antigen-MHC complex is a MHC class II molecule~~s and said antigen is specific for the autoimmune disease further wherein said administration of said complex is in an amount sufficient to expand the population of anti-pathogenic autoreactive TR1 cells which cells express IL-10;~~

~~permitting said TR1 cells to accumulate at the site of said autoimmune disease whereby IL-10 is allowed to accumulate at said site under conditions which result in a reduction of the inflammatory component of the autoimmune disease~~.

16. The composition for the use of Claim 15, wherein said autoreactive T cells are CD4+ TR1 cells.

17. The composition for the use of Claim 16, wherein said CD4+ TR1 cells are characterized by IL-10 and IFNγ expression.

18. The composition for the use of any one of Claims 1 to 17, wherein said biocompatible bioabsorbable nanospheres are formed from one or more biocompatible, bioabsorbable materials.

~~14~~19. The composition for the use of any one of Claims 1 to 2, 4 to 14 and 16 ~~and 11~~ to 1~~3~~8 ~~or the complex of any one of Claims 7 to 10~~, wherein the MHC is MHC I or MHC II or a combination thereof.

~~15~~20. The composition for the use of any one of Claims 1~~, 3 to 6, and 11~~ to ~~14~~19, ~~or the complex of any one of Claims 7 to 10,~~ wherein said autoimmune disorder or disease is pre-diabetes, diabetes, type I diabetes, type II diabetes, allergic asthma, multiple sclerosis, primary biliary cirrhosis, neuromyelitis optica, pemphigus vulgaris, irritable bowel disease, Crohn's disease, colitis, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, Celiac disease, psoriasis, cardiomyopathy, myasthyenia gravis, uveitis, ankylosing spondylitis, Grave's disease, inflammatory myopathy, or anti-phospholipid antibody syndrome.

21. The composition for the use of any one of Claims 1 to 20, wherein the subject is a human patient and the amount of antigen-MHC complexes administered to the patient is 0.0004054 mg/kg to 2.027 mg/kg.

22. The composition for the use of any one of Claims 1 to 4, wherein:

a) the autoimmune disorder or disease is type I diabetes, the antigen is derived from an antigen selected from the group consisting of PPI, IGRP, GAD, or pro-insulin, and the MHC component comprises all or part of HLA-DR; or

b) the autoimmune disorder or disease is multiple sclerosis, the antigen is derived from an antigen selected from the group consisting of MOG, MBP, or PLP, and the MHC component comprises all or part of HLA-DR.

23. A nanoparticle complex comprising:

a) a nanosphere core having a diameter of 4 nm or from 5 to 15 nm; and

b) an autoimmune disorder- or disease-relevant antigen-MHC complex operatively coupled to the nanosphere.

24. The nanoparticle complex of Claim 23, wherein the nanoparticle core comprises a biocompatible core and a biodegradable bioabsorbable coating on the outer surface of the biocompatible core.

25. The nanoparticle complex of Claim 24, wherein the biocompatible core is selected from iron, iron(III) oxide, a polymer, and gold.

26. The nanoparticle complex of Claim 24 or 25, wherein the biodegradable bioabsorbable coating is selected from dextran, mannitol, and poly(ethylene glycol).

27. The nanoparticle complex of any one of Claims 23 to 26, wherein the biocompatible bioabsorbable nanosphere is covalently bound to the MHC group of the antigen-MHC complex, and wherein said binding is optionally via a linker.

28. The nanoparticle complex of any one of Claims 23 to 27, wherein the MHC molecule comprises MHC class I, MHC class II, or a combination thereof.

29. The nanoparticle complex of any one of Claims 23 to 28, wherein more than 50 to 200 antigen-MHC complexes are operatively coupled to the nanosphere.

30. The nanoparticle complex of any one of Claims 23 to 29, wherein the antigen-MHC complexes comprise a plurality of auto-antigen epitopes.

31. The nanoparticle complex of Claim 30, wherein the plurality of auto-antigen epitopes are derived from a single auto-antigen or a plurality of auto-antigens.

32. The nanoparticle complex of any one of Claims 23 to 31, wherein the antigen of the antigen-MHC complex is relevant to an autoimmune disorder or disease selected from pre-diabetes, diabetes, type I diabetes, type II diabetes, allergic asthma, multiple sclerosis, primary biliary cirrhosis,

neuromyelitis optica, pemphigus vulgaris, irritable bowel disease, Crohn's disease, colitis, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, Celiac disease, psoriasis, cardiomyopathy, myasthyenia gravis, uveitis, ankylosing spondylitis, Grave's disease, inflammatory myopathy, and anti-phospholipid antibody syndrome.

33. The nanoparticle complex of any one of Claims 23 to 32, wherein:

a) the autoimmune disorder or disease is type I diabetes, the antigen is derived from an antigen selected from the group consisting of PPI, IGRP, GAD, or pro-insulin, and the MHC component comprises all or part of HLA-DR; or

b) the autoimmune disorder or disease is multiple sclerosis, the antigen is derived from an antigen selected from the group consisting of MOG, MBP, or PLP, and the MHC component comprises all or part of HLA-DR.

34. A method to detect a population of T cells in an antigen specific manner in a subject that has received the composition of any one of Claims 1 to 22 or the complex of any one of Claims 23-33, the method comprising contacting a sample suspected of comprising the T cells with an effective amount of a tetramer complex comprising the antigen-MHC complex, and detecting any population of T cells bound to the tetramer complex.

35. The method of Claim 34, further comprising staining any T cell population using a labeled tetramer complex.

36. The method of Claim 34 or 35, wherein the step of detecting the population of T cells comprises flow cytometry.

37. A method to monitor the expansion of a population of antigen-specific T cells in a subject, the method comprising:

a) contacting an isolated sample from a subject that has received the composition of any one of Claims 1 to 22 or the complex of any one of Claims 23-33 and suspected of containing the population with an effective amount of a tetramer complex comprising the antigen-MHC complexes; and

b) detecting and quantifying the number of antigen-specific T cells bound to the tetramer complex.

38. The method of Claim 37, wherein contacting an isolated sample from the subject with an effective amount of a tetramer complex comprising the antigen MHC-complexes and detecting and quantifying the number of antigen-specific T cells bound to the tetramer complex is first perfomed prior to the subject receiving said composition.

39. The method of Claim 38, further comprising comparing the number of antigen-specific T cells before and after the subject has received said composition.

40. The method of any one of Claims 34 to 39, further comprising staining any tetramer complex prior to step b).

41. The method of any one of Claims 37 to 40, wherein the step of quantifying the number of antigen-specific T cells of step b) comprises the use of flow cytometry.

~~16~~42. Use of a composition of high particle density nanoparticles comprising ~~from 10 to 1000~~ antigen-MHC complexes covalently coupled to a biocompatible bioabsorbable nanosphere in the preparation of a medicament for diagnosis, preventing or treating an autoimmune disorder in a subject, wherein the composition of high density nanoparticles comprises higher numbers of smaller nanoparticles for administration to ~~a~~ the subject ~~in an amount sufficient~~ to expand anti-pathogenic autoreactive T cells.

~~17~~43. Use of a composition of high particle density nanoparticles comprising ~~from 10 to 1000~~ antigen-MHC complexes covalently coupled to a biocompatible bioabsorbable nanosphere in the preparation of a medicament for ~~use in~~ expanding and/or developing populations of anti-pathogenic autoreactive T-cells in a subject, wherein the composition of high density nanoparticles comprises higher numbers of smaller nanoparticles for administration to the subject ~~in amount and frequency sufficient~~ to expand said populations.

44. Use of a composition of high particle density nanoparticles comprising antigen-MHC complexes covalently coupled to a biocompatible nanosphere in the preparation of a medicament for inhibiting the onset of an auto-immune disease in a subject, wherein the MHC of the antigen-MHC complexes comprise MHC class II molecules, and wherein the high particle density composition comprises higher numbers of smaller nanoparticles for administration to the subject to expand anti-pathogenic autoreactive T cells.

45. Use of a composition of high particle density nanoparticles comprising antigen-MHC complexes covalently coupled to a biocompatible nanosphere in the preparation of a medicament for treating

the inflammatory component of an autoimmune disease in a subject suffering from an autoimmune disease having an inflammatory component and said antigen is specific for the autoimmune disease, wherein the high particle density composition comprises higher numbers of smaller nanoparticles for administration to the subject to expand anti-pathogenic autoreactive T cells which express IL-10 permitting said TR1 cells to accumulate at the site of said autoimmune disease whereby IL-10 is allowed to accumulate at said site under conditions which result in a reduction of the inflammatory component of the autoimmune disease.

~~18~~46. The use of any one of Claims ~~16 or of Claim 17~~42 to 45, wherein the antigens of the antigen-MHC complexes comprise a plurality of auto-antigen epitopes ~~are contained in said auto-antigen epitope-MHC biocompatible bioabsorbable nanosphere complexes~~.

~~19~~47. The use of Claim ~~18~~46, wherein said plurality of auto-antigen epitopes are derived from a single auto-antigen or a plurality of auto-antigens.

~~20~~48. The use of any one of Claims ~~19~~42 to 47, wherein the expanded population of anti-pathogenic autoreactive T-cells are antigen specific but suppress in a non-antigen-specific manner.

~~21~~49. The use of any one of Claims ~~17, 19 or 20~~42 to 48, wherein ~~the administration of~~ said biocompatible absorbable nanosphere complex upon administration depletes the population of cognate or non-cognate pathogenic autoreactive T cells.

50. The use of any one of Claims 42 to 49, wherein the nanoparticles comprises biocompatible cores and a biodegradable bioabsorbable coating on the outer surface of said cores.

51. The use of Claim 50, wherein said biocompatible cores is selected from iron, iron (III) oxide, a polymer, and gold.

52. The use of Claim 50, wherein said biodegradable bioabsorbable coating is selected from dextran, mannitol, and poly(ethylene glycol).

53. The use of any one of Claims 42 to 52, wherein said covalent coupling is via a linker.

~~22~~54. The ~~U~~use of any one of Claims 42 to 53, wherein said autoreactive T cells are CD4+ TR1 cells ~~composition comprising from 10 to 1000 antigen-MHC complexes covalently coupled to a biocompatible nanosphere in the preparation of a medicament for inhibiting the onset of an auto-immune disease for administration to a subject in an amount sufficient to expand anti-pathogenic autoreactive T cells wherein the MHC molecule comprises MHC class II molecules~~.

~~23~~55. The use ~~method~~ of Claim ~~22~~54, wherein said ~~autoreactive T-cells are CD4+ TR1 cells and optionally wherein the~~ CD4+ TR1 cells are characterized by IL-10 and IFNγ expression.

~~24~~56. The ~~U~~use of any one of Claims 42 to 43 and 45 to 55 ~~a composition comprising from 10 to 1000 antigen-MHC complexes covalently coupled to a biocompatible nanosphere in the preparation of a medicament for treating the inflammatory component of an autoimmune disease for administration to a subject suffering from an autoimmune disease having an inflammatory component an antigen-MHC complex operatively coupled to a biocompatible nanosphere~~ wherein the MHC ~~molecule comprises~~ of the antigen-MHC complex is a MHC class II molecule~~s and said antigen is specific for the autoimmune disease further wherein said administration of said complex is in an amount sufficient to expand the population of anti-pathogenic autoreactive TR1 cells which cells express IL-10;~~

~~permitting said TR1 cells to accumulate at the site of said autoimmune disease whereby IL-10 is allowed to accumulate at said site under conditions which result in a reduction of the inflammatory component of the autoimmune disease~~.

57. The use of Claim 56, wherein said autoreactive T-cells are CD4+ TR1 cells.

58. The use of Claim 57, wherein said CD4+ TR1 cells are characterised by IL-10 and IFNγ expression.

59. The use of any one of Claims 42 to 58, wherein said biocompatible bioabsorbable nanospheres are formed from one or more biocompatible, bioabsorbable materials.

~~25~~60. The use of any one of Claims ~~16~~42 to ~~24~~3, 45 to 55 and 57 to 59, wherein the MHC is MHC I or MHC II or a combination thereof.

~~26~~61. The use of any one of Claims ~~16 to 25~~42 to 60, wherein said autoimmune disease is pre-diabetes, diabetes,type I diabetes, type II diabetes, allergic asthma, multiple sclerosis, primary biliary cirrhosis, neuromyelitis optica, pemphigus vulgaris, irritable bowel disease, Crohn's disease, colitis, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, Celiac disease, psoriasis, cardiomyopathy, myasthyenia gravis, uveitis, ankylosing spondylitis, Grave's disease, inflammatory myopathy, or anti-phospholipid antibody syndrome.

62. The use of any one of Claims 42 to 61, wherein the subject is a human patient and the amount of antigen-MHC complexes for administration to the patient is 0.0004054 mg/kg to 2.027 mg/kg.

63. The use of any one of Claims 42 to 45, wherein:

a) the autoimmune disorder or disease is type I diabetes, the antigen is derived from an antigen selected from the group consisting of PPI, IGRP, GAD, or pro-insulin, and the MHC component comprises all or part of HLA-DR; or

b) the autoimmune disorder or disease is multiple sclerosis, the antigen is derived from an antigen selected from the group consisting of MOG, MBP, or PLP, and the MHC component comprises all or part of HLA-DR.