# METHODS FOR TREATING AUTOIMMUNE DISEASE USING BIOCOMPATIBLE BIOABSORBABLE NANOSPHERES

## Cross-Reference to Related Applications

**[0001]** This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 61/387,873 filed September 29, 2010 ~~which application is incorporated herein by reference in its entirety~~.

## Field of the Invention

**[0002]** This invention is directed to compositions and methods related to immunology and medicine. In particular, this invention is related to diagnostics and therapeutics for the diagnosis and treatment of autoimmune conditions, particularly diabetes.

## State of the Art

**[0003]** Antigen vaccination can be used for the induction of T-cell tolerance in autoimmunity. Administration of autoantigenic proteins or peptides in solution can blunt the initiation and/or progression of autoimmunity in experimental models of autoimmune disease (Wraith et al., 1989; Metzler and Wraith, 1993; Liu and Wraith, 1995; Anderton and Wraith, 1998; Karin et al., 1994). However, limited clinical trials in humans employing similar strategies have almost invariably met with failure (Weiner, 1993; Trentham et al., 1993; McKown et al., 1999; Pozzilli et al., 2000; Group, D.P.T.-T.D.S. 2002; Kappos et al., 2000; Bielekova et al., 2000). This suggests that the principles guiding the choice and conditions of treatment are poorly defined and, as a result, inadequate for human application.

**[0004]** Spontaneous organ-specific autoimmune disorders result from complex responses against numerous epitopes in multiple antigens that arise spontaneously in a stochastic and often unpredictable sequence. This complexity is compounded by the fact that lymphocyte clones recognizing identical epitopes engage antigen/major histocompatibility complex (MHC) molecules within a broad range of avidities, the strength of which correlates with pathogenic potential (Amrani et al., 2000; Santamaria, 2001; Liblau et al., 2002). Consequently, the outcome of any immunization strategy for the prevention of autoimmunity is likely to be

bioabsorbable coating. The nanosphere may further comprise a covering or shell of other molecules that can be easily coupled to the antigen-MHC complex (e.g, streptavidin or avidin or other know molecules used to attach moieties to nanospheres). In certain aspects, a biocompatible, bioabsorbable nanosphere comprises a material selected from the group consisting of, for example, iron III oxide, tricalcium phosphate,chromium, gallium, as well as biocompatible, bioabsorbable polymers such as PGLA, PLLA, PGA, PDLLA, PCL, PDLGA, PLDLA, PLC (all of which are available from Zeus, 3737 Industrial Blvd, Orangeburg, SC, 29118 USA under the tradename Absorv$^{TM}$), hylaurinic acid, alginate, polyhydroxyalkanoates, and the like. In further aspects, a biocompatible bioabsorbable nanosphere is a metal or magnetizable or superparamagnetic nanosphere. The biocompatible, bioabsorbable nanosphere may further comprise a biodegradable coating formed from dextran; poly(ethylene glycol); poly(ethylene oxide); mannitol; poly(hydroxalkanoate)s of the PHB-PHV class; and other modified poly(saccharides) such as starch, cellulose and chitosan.

**[0017]** Certain aspects of the invention include methods and compositions concerning antigenic compositions including segments, fragments, or epitopes of polypeptides, peptides, nucleic acids, carbohydrates, lipids and other molecules that provoke or induce an antigenic or immune response, generally referred to as antigens. In particular aspects, the antigen is derived from, is a mimic of, or is an autoreactive antigen and/or complexes thereof.

**[0018]** Peptide antigens include, but are not limited to hInsB$_{10-18}$ (HLVEALYLV (SEQ ID NO:1)), hIGRP$_{228-236}$ (LNIDLLWSV (SEQ ID NO:2)), hIGRP$_{265-273}$ (VLFGLGFAI (SEQ ID NO:3)), IGRP$_{206-214}$ (VYLKTNVFL (SEQ ID NO:4)), NRP-A7 (KYNKANAFL (SEQ ID NO:6)), NRP-14 (KYNIANVFL (SEQ ID NO:7)), NRP-V7 (KYNKANVFL (SEQ ID NO:8)), YAI/D$^{b}$ (FQDENYLYL (SEQ ID NO:9)) and/or INS B$_{15-23}$ (LYLVCGERG (SEQ ID NO:10)), as well as peptides and proteins disclosed in U.S. Publication 20050202032~~, which is incorporated herein by reference in its entirety~~.

**[0019]** In certain aspects, a peptide antigen for treatment of T1D is GAD65$_{114-123}$, VMNILLQYVV (SEQ ID NO:14); GAD65$_{536-545}$, RMMEYGTTMV (SEQ ID NO:15); GFAP$_{143-151}$, NLAQTDLATV (SEQ ID NO:16); GFAP$_{214-222}$, QLARQQVHV (SEQ ID NO:17); IA-2$_{172-180}$, SLSPLQAEL (SEQ ID NO:18); IA-2$_{482-490}$, SLAAGVKLL (SEQ ID NO:19); IA-2$_{805-813}$,

Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterilized solution thereof. One such method of sterilization of the solution is sterile filtration, however, this invention is meant to include any method of sterilization that does not significantly decrease the anti-pathogenic properties of the peptide/MHC/nanosphere complexes. Methods of sterilization that involve intense heat and pressure, such as autoclaving, may compromise the tertiary structure of the complex, thus significantly decreasing the anti-pathogenic properties of the peptide/MHC/nanosphere complexes.

**[0087]** Administration of the compositions according to the present invention will typically be via any common route. This includes, but is not limited to orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection. In certain embodiments, a vaccine composition may be inhaled (e.g., U.S. Pat. No. 6,651,655~~, which is specifically incorporated by reference in its entirety~~).

**[0088]** An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, i.e., the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the result and/or protection desired. Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

immunogenicity or ability to interact with other cells of the immune system when in the context of an MHC/nanosphere complex.

**[0097]** Peptides of the invention include any autoreactive peptide. Autoreactive peptides include, but are not limited to $hInsB_{10-18}$ (HLVEALYLV (SEQ ID NO:1)), $hIGRP_{228-236}$ (LNIDLLWSV (SEQ ID NO:2)), $hIGRP_{265-273}$ (VLFGLGFAI (SEQ ID NO:3)), $IGRP_{206-214}$ (VYLKTNVFL (SEQ ID NO:4)), $hIGRP_{206-214}$ (VYLKTNLFL (SEQ ID NO:5)), NRP-A7 (KYNKANAFL (SEQ ID NO:6)), NRP-I4 (KYNIANVFL (SEQ ID NO:7)), NRP-V7 (KYNKANVFL (SEQ ID NO:8)), YAI/$D^b$ (FQDENYLYL (SEQ ID NO:9)) and/or INS $B_{15-23}$ (LYLVCGERG (SEQ ID NO: 10)), as well as peptides and proteins disclosed in U.S. Publication 20050202032~~, which is incorporated herein by reference in its entirety~~. Other peptides that may be used in conjunction with invention as autoreactive peptides or as control peptides include, but are not limited to INS-I9 (LYLVCGERI (SEQ ID NO:11)), TUM (KYQAVTTTL (SEQ ID NO:12)), and G6Pase (KYCLITIFL (SEQ ID NO:13)). In certain aspects, 1, 2, 3, 4, 5, 6 or more peptides can be used. Examples of peptides that can be used in conjunction with the present invention also include those provided in Table 1. These peptides may be associated with specific nanosphere/MHC molecules or multiple peptides may be associated with a common nanosphere and one or more MHC molecule. Administration of combinations of these peptides includes administering a population of nanospheres having multiple peptides attached and/or administering multiple nanosphere populations each having a specific peptide attached or a combination of such nanospheres that includes nanospheres with 1, 2, 3, 4, 5, 6, or more peptides attached to 1, 2, 3, 4, 5, 6, or more nanospheres.TABLE 1A HLA class I-restricted epitopes for T1D

| Antigen | Epitope | HLA | Amino Acid Sequence | Comments | References |
|---|---|---|---|---|---|
| GAD65 | 114-123 | A2 | VMNILLQYVV (SEQ ID NO:14) | Reactivity detected in immunized HHD mice and T1D patients | Blancou *et. al.* 2007, Panina-Bordignon *et al.* 1995, Mallone *et al.* 2007 |
| | 536-545 | A2 | RMMEYGTTMV (SEQ ID NO:15) | Reactivity detected in plasmid-immunized HHD mice and T1D patients | Blancou *et. al.* 2007 |
| GFAP | 143-151 | A2 | NLAQTDLATV | Reactivity detected in T1D patients | Ouyang *et. al.* |

sequences, respectively, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity (e.g., immunogenicity). The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

**[0106]** It is contemplated that in compositions of the invention, there is between about 0.001 mg and about 10 mg of total protein per ml. Thus, the concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 50, 100 μg/ml or mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% may be peptide/MHC/nanosphere complex.

**[0107]** The present invention contemplates the administration of a peptide/MHC/nanosphere complex to effect a diagnosis, treatment or preventative therapy against the development of a disease or condition associated with autoimmune responses.

**[0108]** In addition, U.S. Pat. No. 4,554,101 (Hopp), ~~which is incorporated herein by reference,~~ teaches the identification and preparation of epitopes from primary amino acid sequences on the basis of hydrophilicity. Through the methods disclosed in Hopp, one of skill in the art would be able to identify potential epitopes from within an amino acid sequence and confirm their immunogenicity. Numerous scientific publications have also been devoted to the prediction of secondary structure and to the identification of epitopes, from analyses of amino acid sequences (Chou & Fasman, 1974a,b; 1978a,b; 1979). Any of these may be used, if desired, to supplement the teachings of Hopp in U.S. Pat. No. 4,554,101.

### 2. Other Antigenic Components

**[0109]** Molecules other than peptides can be used as antigens or antigenic fragments in complex with MHC molecules, such molecules include, but are not limited to carbohydrates, lipids, small molecules, and the like. Carbohydrates are major components of the outer surface of a variety of cells. Certain carbohydrates are characteristic of different stages of differentiation and very often these carbohydrates are recognized by specific antibodies. Expression of distinct carbohydrates can be restricted to specific cell types. Autoantibody responses to endometrial and serum antigens have been shown to be a common feature of endometriosis. There has been described a serum autoantibody response in endometriosis to a number of previously identified antigens, including 2-Heremans Schmidt glycoprotein and carbonic anhydrase, that is specific for a carbohydrate epitope (Yeaman et al., 2002).

### C. Substrates/Nanospheres

**[0110]** In certain aspect, antigen/MHC complexes are operatively coupled to a substrate. A substrate can be in the form of a nanoparticle comprising a biocompatible, bioabsorbable material. A substrate can also be in the form of a nanoparticle such as those described previously in US application 12/044,435 ~~which is herein incorporated by reference in its entirety~~. Nanoparticles can have a structure of variable dimension and known variously as a nanosphere or biocompatible biodegradable nanosphere. Such particulate formulations containing an antigen/MHC complex can be formed by covalent or non-covalent coupling of the complex to the nanosphere.

**[0111]** The nanospheres typically consist of a substantially spherical core and optionally one or more layers. The core may vary in size and composition. In addition to the core, the nanosphere may have one or more layers to provide functionalities appropriate for the applications of interest. The thicknesses of layers, if present, may vary depending on the needs of the specific applications. For example, layers may impart useful optical properties.

**[0112]** Layers may also impart chemical or biological functionalities, referred to herein as chemically active or biologically active layers, and for these functionalities the layer or layers may typically range in thickness from about 0.001 micrometers (1 nanometer) to about 10 micrometers or more (depending on the desired nanosphere diameter), these layers typically being applied on the outer surface of the nanosphere.

particular, chain-type molecules with amphiphilic character, such as phospholipids or derivatised polysaccharides, which can be crosslinked with each other, are useful. The absorption of these molecules on the surface can be achieved by coincubation. The binding between affinity molecule and substrate or nanosphere can also be based on non-covalent, self-organising bonds. One example thereof involves simple detection probes with biotin as linking molecule and avidin- or strepdavidin-coupled molecules.

**[0128]** Protocols for coupling reactions of functional groups to biological molecules can be found in the literature, for instance in "Bioconjugate Techniques" (Greg T. Hermanson, Academic Press 1996). The biological molecule (e.g., MHC molecule or derivative thereof) can be coupled to the linking molecule, covalently or non-covalently, in line with standard procedures of organic chemistry such as oxidation, halogenation, alkylation, acylation, addition, substitution or amidation. These methods for coupling the covalently or non-covalently bound linking molecule can be applied prior to the coupling of the linking molecule to the substrate or nanosphere or thereafter. Further, it is possible, by means of incubation, to effect a direct binding of molecules to correspondingly pre-treated substrate or nanospheres (for instance by trimethylsilyl bromide), which display a modified surface due to this pre-treatment (for instance a higher charge or polar surface).

### E. Protein Production

**[0129]** The present invention describes polypeptides, peptides, and proteins for use in various embodiments of the present invention. For example, specific peptides and their complexes are assayed for their abilities to elicit or modulate an immune response. In specific embodiments, all or part of the peptides or proteins of the invention can also be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young (1984); Tam et al. (1983); Merrifield (1986); and Barany and Merrifield (1979)~~, each incorporated herein by reference~~. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.

perforin-deficient NOD mice will show that restoration of normoglycemia by pMHC class II-nanospheres is IL-10- and perforin-dependent. It is further contemplated that IFNγ is necessary for development of the Tr1 cells that pMHC-nanospheres may expand, but not for their suppressive activity. The cognate autoreactive T-cells that may expand in response to pMHC-nanosphere therapy in IFNγ-/- mice may have a Th2 phenotype (producing IL-4 and IL-10 in response to peptide challenge ex vivo), whereas those expanding in IL-10-/- mice may produce IFNγ and IL-4 but no IL-10. These results would indicate that diabetes reversal is mediated by Tr1-like cells.

Example 4

**T-CELL EXPANSION USING IGRP/Kd-PLGA NANOSPHERES.**

**[0203]** IGRP206-214/Kd-PLGA nanospheres induce the activation of cognate CD8+ T-cells *in vitro*. This assay was performed essentially as described in Tsai et al., Immunity (2010) 32, 568-680 ~~which is herein incorporated by reference~~. Briefly, purified naïve IGRP206-214/Kd-specific T cell receptor (TCR)-transgenic CD8+ T-cells from 8.3-NOD mice were cultured with IGRP206-214/Kd-PLGA nanospheres for 48h. The supernatants were assayed for IFN-gamma. The cells were pulsed with 1 μCi of [3H]-thymidine and harvested. The CPM and IFN-gamma was measured in relation to the number of nanospheres per well. This is depicted in Figure 9. Figure 8 depicts a magnified image of the IGRP/Kd conjugated PLGA particles. The PLGA particles were made according to the following:Concentration: 100 nm (PLGA: 20 mg/mL in 1.5 mL).
DLS size: 154.72 nm
pMHC Conjugation: conjugated 5 mg IGRP/Kd with 2 mL PLGA particles
Final volume: 2 mL

**REFERENCES**

~~The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference in their entirety.~~