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(54) **CONTROL OF ANTIBODY RESPONSES TO SYNTHETIC NANOCARRIERS**

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(57) **ABSTRACT**

Disclosed are synthetic nanocarrier compositions that comprise B cell antigen for desired antibody production and an off-target response attenuating polymeric coating as well as related methods.

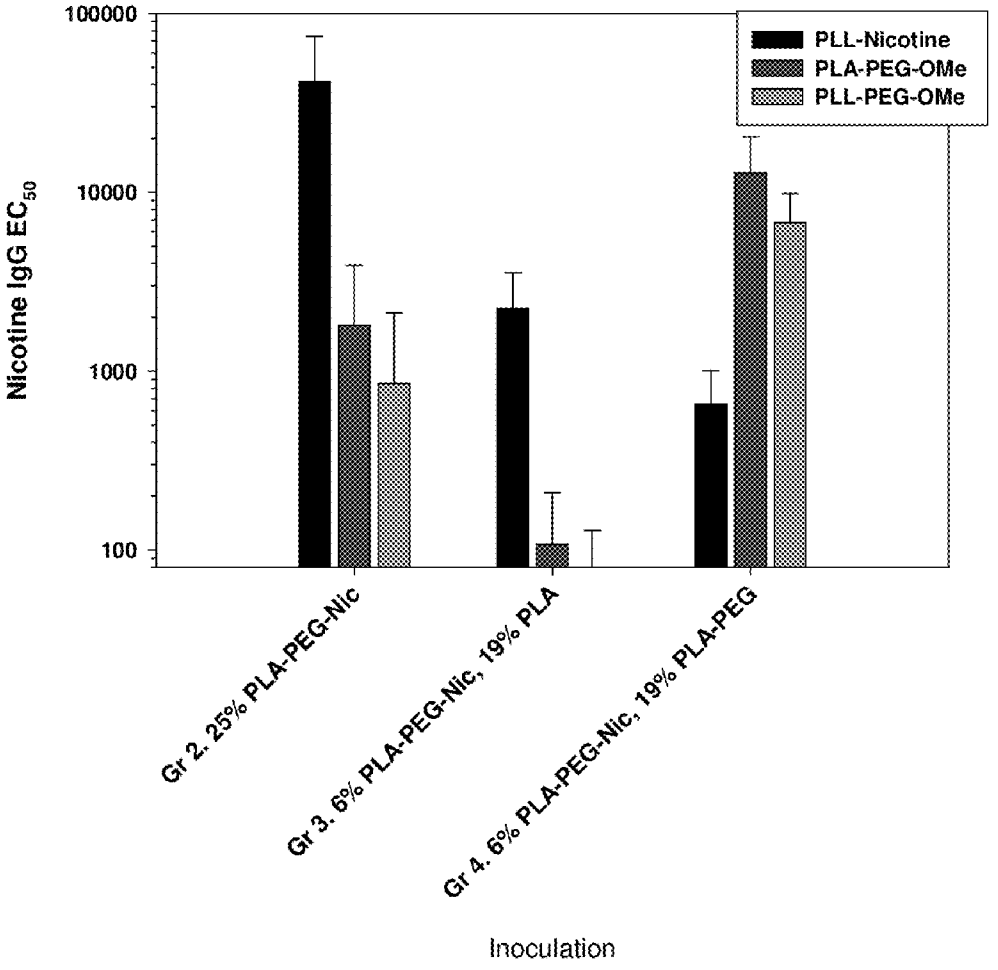

100000
10000
1000
100
Nicotine IgG EC50
PLL-Nicotine
PLA-PEG-OMe
PLL-PEG-OMe
Gr 2. 25% PLA-PEG-Nic
Gr 3. 6% PLA-PEG-Nic, 19% PLA
Gr 4. 6% PLA-PEG-Nic, 19% PLA-PEG
Inoculation

Fig. 1

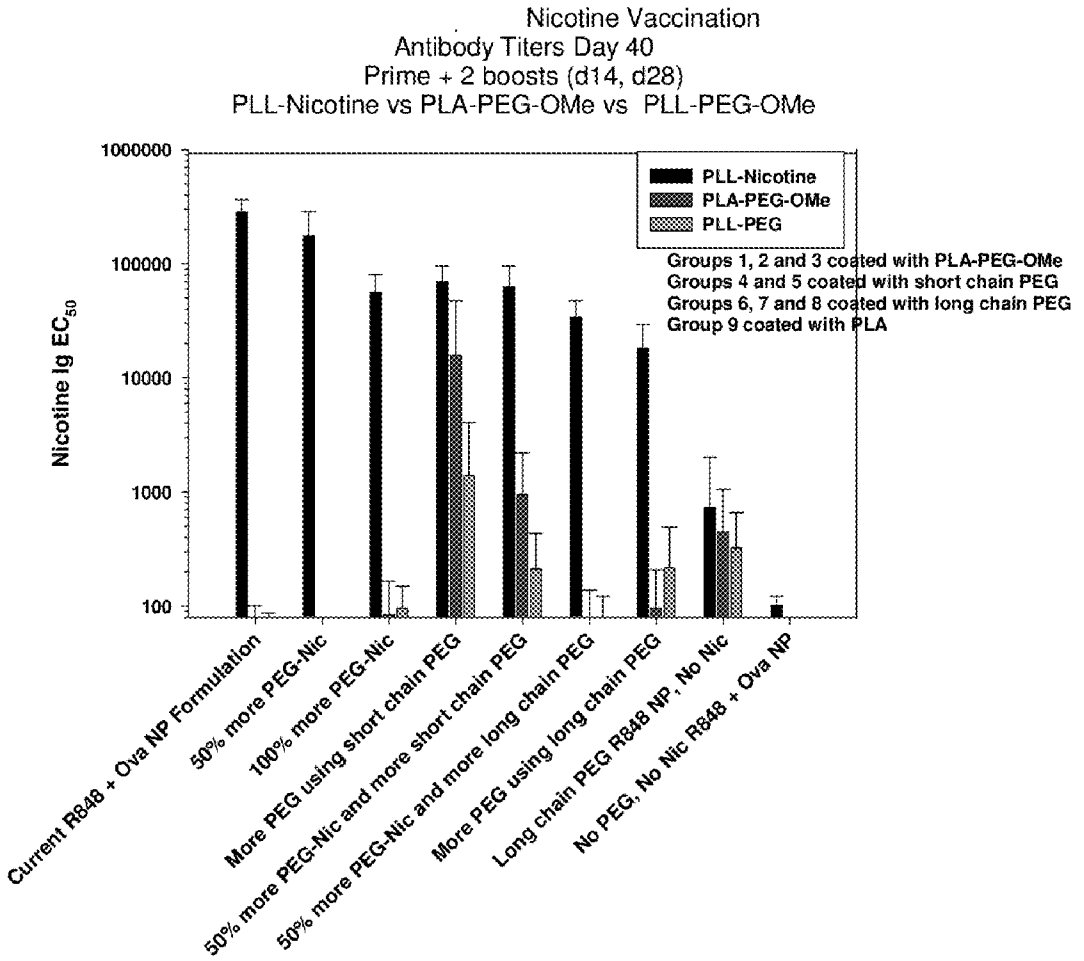

Nicotine Vaccination
Antibody Titers Day 40
Prime + 2 boosts (d14, d28)
PLL-Nicotine vs PLA-PEG-OMe vs PLL-PEG-OMe
Nicotine Ig EC50
1000000
100000
10000
1000
100
PLL-Nicotine
PLA-PEG-OMe
PLL-PEG
Groups 1, 2 and 3 coated with PLA-PEG-OMe
Groups 4 and 5 coated with short chain PEG
Groups 6, 7 and 8 coated with long chain PEG
Group 9 coated with PLA
Current R848 + Ova NP Formulation
50% more PEG-Nic
100% more PEG-Nic
More PEG using short chain PEG
50% more PEG-Nic and more short chain PEG
50% more PEG-Nic and more long chain PEG
More PEG using long chain PEG
Long chain PEG R848 NP, No Nic
No PEG, No Nic R848 + Ova NP

Fig. 2

CONTROL OF ANTIBODY RESPONSES TO SYNTHETIC NANOCARRIERS

RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119 of U.S. provisional application 61/513,496, 61/513,526 and 61/513,527, each filed Jul. 29, 2011, the entire contents of each of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to synthetic nanocarrier compositions that comprise an off-target response attenuating polymeric coating, and related methods, such as for treating diseases or conditions in which generating an immune response against a B cell antigen is desirable.

BACKGROUND OF THE INVENTION

[0003] Anti-carrier antibody generation by a nanocarrier vaccine is an off-target side effect that may have direct unintended or undesirable impacts on pharmaceutical or biomedical formulations of related compositions, and may interfere with the generation of desired anti-B cell antigen antibodies. Therefore, improved compositions and therapeutic methods to avoid or minimize undesirable anti-carrier effects are needed to provide improved therapies for diseases and conditions in which generating an immune response against a B cell antigen is desirable.

SUMMARY OF THE INVENTION

[0004] In one aspect, a composition comprising a population of synthetic nanocarriers, wherein the synthetic nanocarriers comprise a B cell antigen and an off-target response attenuating polymeric coating is provided. In one embodiment, the B cell antigen is coupled to the synthetic nanocarrier.

[0005] In another aspect, a composition comprising a population of synthetic nanocarriers, wherein the synthetic nanocarriers comprise (i) a B cell antigen and (ii) a coating comprising one or more polymers present at at least a portion of the surface of the synthetic nanocarriers is provided. In one embodiment, the B cell antigen is coupled to the synthetic nanocarrier.

[0006] In an embodiment of any of the compositions provided, the synthetic nanocarriers generate on average across the population of synthetic nanocarriers an antibody response against the B cell antigen that is at least two-fold greater than an off-target antibody response. In another embodiment, the antibody response against the B cell antigen is at least five-fold greater than the off-target antibody response. In another embodiment, the antibody response against the B cell antigen is at least ten-fold greater than the off-target antibody response. In another embodiment, the antibody response against the B cell antigen is at least 25-fold greater than the off-target antibody response. In another embodiment, the antibody response against the B cell antigen is at least 50-fold greater than the off-target antibody response. In another embodiment, the antibody response against the B cell antigen is at least 100-fold greater than the off-target antibody response. In one embodiment, the off-target antibody response is an undesired antibody response not specific to the B cell antigen. In another embodiment, the off-target antibody response is an antibody response again the synthetic nanocarrier. In another embodiment, the off-target antibody response is an antibody response again the coating. In another embodiment, the off-target antibody response is an antibody response against a polymer of the coating. In another embodiment, the off-target antibody response is an IgG or IgA antibody response. In another embodiment, the desired antibody response is also an IgG or IgA antibody response, respectively. In another embodiment, the off-target antibody response is an IgG antibody response and the desired antibody response is also an IgG antibody response. In another embodiment, the off-target antibody response is an IgA antibody response and the desired antibody response is also an IgA antibody response. In another embodiment, the antibody responses are each measured as an antibody titer with an ELISA. In another embodiment, the antibody titer is an IgG or IgA titer (EC50).

[0007] In one embodiment, the B cell antigen is coupled to the coating. In another embodiment, the B cell antigen is coupled to one or more polymers of the coating. In another embodiment, the B cell antigen is coupled to another part of the synthetic nanocarriers.

[0008] In yet another embodiment, the off-target antibody response is an undesired antibody response not specific to the B cell antigen. In another embodiment, the off-target antibody response is an antibody response against a polymer (or portion thereof) of the nanocarrier or its coating.

[0009] In one embodiment, the antibody response against the B cell antigen is at least five-fold greater than the antibody response against a polymer of the off-target response attenuating polymeric coating. In another embodiment, the antibody response is at least ten-fold greater. In still another embodiment, the antibody response is at least 25-fold greater. In yet another embodiment, the antibody response is at least 50-fold greater. In a further embodiment, the antibody response is at least 100-fold greater.

[0010] In one embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of greater than 2000 g/mole. In another embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of greater than 3000 g/mole. In yet another embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of greater than 4000 g/mole. In still another embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of greater than 5000 g/mole. In another embodiment, the off-target response attenuating polymeric coating comprises a polymer with a weight average or number average molecular weight of between 3500 g/mole and 5000 g/mole In a further embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of 5000 g/mole. In another embodiment, the B cell antigen is coupled to the polymer. In one embodiment of any of the foregoing embodiments, the molecular weight is the weight average molecular weight. In another embodiment of any of the foregoing embodiments, the molecular weight is the number average molecular weight. In still another embodiment of any of the foregoing embodiments where the polymer does not comprise polyethylene glycol, the molecular weight is the weight average molecular weight. In yet another embodiment of any of the foregoing embodiments where the polymer does comprise polyethylene glycol, the molecular weight is the number average molecular weight.

[0011] In still another embodiment, the off-target response attenuating polymeric coating comprises another polymer.

This other polymer may be the same type of polymer as the aforementioned polymer or it may be a different type of polymer. In one embodiment, this other polymer has a molecular weight of greater than 2000 g/mole. In another embodiment, this other polymer has a molecular weight of greater than 3000 g/mole. In still another embodiment, this other polymer has a molecular weight of greater than 4000 g/mole. In yet another embodiment, this other polymer has a molecular weight of greater than 5000 g/mole. In still another embodiment, this other polymer has a molecular weight of between 3500 g/mole and 5000 g/mole. In yet another embodiment, this other polymer has a molecular weight of 5000 g/mole. In one embodiment, the B cell antigen is coupled to this other polymer. In another embodiment, the B cell antigen is coupled to this other polymer and the aforementioned polymer. In one embodiment of any of the foregoing embodiments, the molecular weight is the weight average molecular weight. In another embodiment of any of the foregoing embodiments, the molecular weight is the number average molecular weight. In still another embodiment of any of the foregoing embodiments where the polymer does not comprise polyethylene glycol, the molecular weight is the weight average molecular weight. In yet another embodiment of any of the foregoing embodiments where the polymer does comprise polyethylene glycol, the molecular weight is the number average molecular weight.

[0012] In one embodiment, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers is between 0.001 and 1. In another embodiment, the ratio is between 0.01 and 1. In still another embodiment, the ratio is between 0.1 and 1. In yet another embodiment, the ratio is between 0.25 and 1. In a further embodiment, the ratio is between 0.5 and 1. In still a further embodiment, the ratio is between 0.75 and 1. In yet another embodiment, the ratio is between 0.1 and 0.5. In a further embodiment, the ratio is 0.5.

[0013] In one embodiment, the ratio is based on the polymeric coating across the population of synthetic nanocarriers. In another embodiment, the ratio is based on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

[0014] In another embodiment, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the populariton of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers is between 0.001 and 1. In another embodiment, the ratio is between 0.01 and 1. In still another embodiment, the ratio is between 0.1 and 1. In yet another embodiment, the ratio is between 0.25 and 1. In a further embodiment, the ratio is between 0.5 and 1. In still a further embodiment, the ratio is between 0.75 and 1. In yet another embodiment, the ratio is between 0.1 and 0.5. In a further embodiment, the ratio is 0.5.

[0015] In one embodiment, the ratio is based on the polymeric coating across the population of synthetic nanocarriers. In another embodiment, the ratio is based on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

[0016] In still another embodiment, the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the populariton of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers is between 0.001 and 1. In another embodiment, the ratio is between 0.01 and 1. In still another embodiment, the ratio is between 0.1 and 1. In yet another embodiment, the ratio is between 0.25 and 1. In a further embodiment, the ratio is between 0.5 and 1. In still a further embodiment, the ratio is between 0.75 and 1. In yet another embodiment, the ratio is between 0.1 and 0.5. In a further embodiment, the ratio is 0.5.

[0017] In one embodiment, the ratio is based on the polymeric coating across the population of synthetic nanocarriers. In another embodiment, the ratio is based on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

[0018] In one embodiment, the polymer and/or other polymer comprises polyethylene glycol. In another embodiment, the polymer and/or other polymer comprises a polyethyloxazoline. In still another embodiment, the polymer and/or other polymer comprises a polyamino acid, polycarbonate, hydrophilic polyacetal, hydrophilic polyketal, polysaccharide, polypropylene or polyethyleneimine.

[0019] In one embodiment, the B cell antigen comprises a protein, peptide, small molecule or oligosaccharide. In another embodiment, the B cell antigen comprises a cancer antigen, an infection or infectious disease antigen, a non-autoimmune or degenerative disease antigen or an addiction antigen.

[0020] In yet another embodiment, the composition and/or B cell antigen further comprises an additional antigen. In one embodiment, the additional antigen is a T cell antigen. In another embodiment, the T cell antigen is a T helper cell antigen. In yet a further embodiment, the T cell antigen is a T helper cell antigen. In still a further embodiment, the additional antigen is another B cell antigen. In still another embodiment, the one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 20 or more, etc. additional antigens are comprised in the compositions provided herein. In one embodiment, the additional antigens are B cell or T cell antigens or some combination thereof. In another embodiment, all of the additional antigens are B cell antigens.

[0021] In another embodiment, the additional antigen is also coupled to the synthetic nanocarriers. In a further embodiment, the additional antigen is also coupled to the off-target response attenuating polymeric coating of the synthetic nanocarriers. In yet another embodiment, the additional antigen is coupled to another population of synthetic nanocarriers. In still another embodiment, the additional antigen is not coupled to any synthetic nanocarriers.

[0022] In one embodiment, the composition further comprises one or more adjuvants.

[0023] In another embodiment, the composition further comprises one or more pharmaceutically acceptable excipients.

[0024] In another aspect, a dosage form comprising any of the compositions provided is provided.

[0025] In yet another aspect, a vaccine comprising any of the dosage forms provided is provided.

[0026] In still another aspect, a method comprising administering any of the compositions provided herein to a subject

in need thereof is provided. In one embodiment, the subject is a human. In another embodiment, the subject has or is at risk of having cancer. In still another embodiment, the subject has or is at risk of having an infection or infectious disease. In yet another embodiment, the subject has or is at risk of having a non-autoimmune or degenerative disease. In a further embodiment, the subject has or is at risk of having an addiction.

[0027] In another embodiment, any of the compositions provided herein is administered by oral, subcutaneous, pulmonary, intranasal, intradermal, intravenous, transmucosal, intramucosal or intramuscular administration.

[0028] In another aspect, a method comprising producing synthetic nanocarriers that comprise a B cell antigen and an off-target response attenuating polymeric coating and determining the level of antibody response against the B cell antigen and the level of off-target antibody response is provided. In one embodiment, the method further comprises comparing the antibody response against the B cell antigen and the off-target antibody response. The antibody response against the B cell antigen and the off-target antibody response can be determined with any of the methods provided herein. The synthetic nanocarriers may be any of the synthetic nanocarriers described herein.

[0029] In another aspect, a process for producing an off-target response attenuating polymeric coating, comprising the steps of: (a) providing a composition comprising one or more polymers present at at least a portion of the surface of a synthetic nanocarrier; (b) coupling a B cell antigen to said synthetic nanocarrier under conditions where: (i) the molecular weight of the polymers; and/or (ii) the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (iii) the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer not coupled to the B cell antigen; and/or (iv) the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (v) the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen; and/or (vi) the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (vii) the ratio by weight averaged across the population of synthetic nanocarriers of polymer not coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen; are selected such that an antibody response against the B cell antigen is at least two-fold greater than an off-target antibody response. In one embodiment, the antibody response against the B cell antigen and the off-target antibody response are each IgG antibody responses. In another embodiment, they are each IgA antibody responses. In another embodiment, these antibody responses are measured as antibody titers (EC50) with an ELISA.

[0030] In one embodiment, the ratio is based on the polymeric coating across the population of synthetic nanocarriers. In another embodiment, the ratio is based on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

[0031] In one embodiment, the molecular weight, ratio of average number and/or ratio by weight of the one or more polymers is as defined herein. In another embodiment, the molecular weight is the weight average molecular weight. In another embodiment of any of the foregoing embodiments, the molecular weight is the number average molecular weight. In still another embodiment of any of the foregoing embodiments where the polymer does not comprise polyethylene glycol, the molecular weight is the weight average molecular weight. In yet another embodiment of any of the foregoing embodiments where the polymer does comprise polyethylene glycol, the molecular weight is the number average molecular weight.

[0032] In another aspect, any of the compositions, dosage forms or vaccines provided herein can be used for therapy or prophylaxis.

[0033] In still another aspect, any of the compositions, dosage forms or vaccines provided herein can be used for any of the methods provided herein.

[0034] In yet another aspect, any of the compositions, dosage forms or vaccines provided herein can be used in vaccination.

[0035] In a further aspect, any of the compositions, dosage forms or vaccines provided herein can be for use in a method of therapy or prophylaxis of cancer, an infection or infectious disease, a non-autoimmune or degenerative disease or an addiction.

[0036] In still a further aspect, any of the compositions, dosage forms or vaccines provided herein can be for use in a method of therapy or prophylaxis comprising administration by oral, subcutaneous, pulmonary, intranasal, intradermal, intravenous, transmucosal, intramucosal or intramuscular administration.

[0037] In another aspect, a use of any of the compositions provided herein for the manufacture of a medicament, for example a vaccine, for use in any of the methods provided herein is provided.

BRIEF DESCRIPTION OF THE FIGURES

[0038] FIG. **1** shows the anti-nicotine antibodies (target or desired antibodies) and anti-PEG antibodies (off-target or undesired antibodies) at day 40 after inoculation.

[0039] FIG. **2** shows the anti-nicotine antibody titers and anti-PEG antibody titers following a prime and two-boost inoculation schedule.

DETAILED DESCRIPTION OF THE INVENTION

[0040] Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified materials or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting of the use of alternative terminology to describe the present invention.

[0041] All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety for all purposes.

[0042] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a polymer" includes a mixture of two or more such molecules or a mixture of differing molecular weights of a single polymer species, reference to "a synthetic nanocarrier" includes a mixture of two or more such synthetic nanocarriers or a plurality of such synthetic nanocarriers, reference to "a DNA molecule" includes a mixture of two or more such DNA molecules or a plurality of such DNA molecules, reference to "an adjuvant" includes mixture of two or more such adjuvant molecules or a plurality of such adjuvant molecules, and the like.

[0043] As used herein, the term "comprise" or variations thereof such as "comprises" or "comprising" are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) but not the exclusion of any other integer or group of integers. Thus, as used herein, the term "comprising" is inclusive and does not exclude additional, unrecited integers or method/process steps.

[0044] In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of" or "consisting of". The phrase "consisting essentially of" is used herein to require the specified integer(s) or steps as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) alone.

A. INTRODUCTION

[0045] Hapten-carrier conjugates are commonly employed constructs for vaccine formulation. A well-known phenomenon related to their use is the creation, or augmentation, of an immune response to the carrier (e.g., anti-carrier antibodies, which are also referred to herein as undesired or off-target antibodies). The anti-carrier response is often of concern as it is not the intended effect of the vaccine and it may relate to undesirable side effects. In the similar case of nanocarrier vaccine formulations, such as biocompatible synthetic nanocarriers presenting an antigen, an anti-carrier effect may also be observed, such as initiated or enhanced undesired antibody generation to the synthetic components of the nanocarrier. In the case of nanocarriers which contain synthetic components, even those with an extended history of safe medical use in humans (e.g., PLGA, PLA, or PEG), anti-carrier effects can result and attenuate the intended vaccine response or alter the vaccine's immune response to those components in other medical applications. It is, therefore, valuable to identify means to formulate nanocarrier vaccines such that the anti-carrier effect is attenuated or absent.

[0046] The inventors have unexpectedly and surprisingly discovered that the problems and limitations noted above can be overcome by practicing the invention disclosed herein. The inventors believe that the invention provided herein is the first of its kind to offer the ability to optimize a target antibody response specific for a B cell antigen of a synthetic nanocarrier composition while attenuating an off-target anti-carrier antibody response. Prior studies have not addressed the design of synthetic nanocarriers relative to optimizing humoral immune responses. Specifically, the inventors have discovered that nanocarriers can be rationally designed as a function of B cell antigen content and/or polymer molecular weights or composition to optimize target antibody generation to the B cell antigen and minimize or eliminate off-target antibody generation. In particular, the inventors have unexpectedly discovered that it is possible to provide compositions with improved target antibody response versus off-target antibody response, and related methods, that comprise a population of synthetic nanocarriers, wherein the synthetic nanocarriers comprise (i) a B cell antigen and (ii) an off-target response attenuating polymeric coating, wherein the synthetic nanocarriers generate on average across the population of synthetic nanocarriers an antibody response against the B cell antigen that is at least two-fold greater than an off-target antibody response. In one embodiment, the respective antibody responses are measured as an antibody titer (e.g., IgG or IgA EC50) with ELISA. In one embodiment, the off-target antibody response is an undesired antibody response against the synthetic nanocarrier or a component thereof not specific to the B cell antigen. In another embodiment, the off-target antibody response is an antibody response against a polymer (or portion thereof) of the synthetic nanocarrier, such as a polymer (or portion thereof) of the coating. The B cell antigen may be coupled to the off-target response attenuating polymeric coating. In another embodiment, the B cell antigen is not coupled to the off-target response attenuating polymeric coating but is coupled to the synthetic nanocarrier. In embodiments, the B cell antigen or portion thereof is present at the surface of the synthetic nanocarrier.

[0047] Preferably, in one embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole given as the weight average molecular weight or number average molecular weight. In another embodiment, the off-target response attenuating polymeric coating comprises a polymer with a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. The B cell antigen may be coupled to the polymer, another polymer or to another portion of the synthetic nanocarrier that is not the coating. In another embodiment, wherein the B cell antigen is coupled to the other polymer, the other polymer has a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole given as a weight average molecular weight or as a number average molecular weight. In another embodiment, the other polymer has a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. In a certain preferred embodiment, the polymer and other polymer both have a molecular weight of 5000 g/mole given as a weight average molecular weight or a number average molecular weight. In still another embodiment of any of the foregoing embodiments where the polymer does not comprise polyethylene glycol, the molecular weight is the weight average molecular weight. In yet another embodiment of any of the foregoing embodiments where the polymer does comprise polyethylene glycol, the molecular weight is the number average molecular weight.

[0048] In another preferred embodiment, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average

number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, or the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers is between 0.001 and 1, 0.01 and 1, 0.1 and 1, 0.25 and 1, 0.5 and 1, 0.75 and 1, 0.01 and 0.75, 0.01 and 0.5, 0.01 and 0.25, 0.1 and 0.75, 0.1 and 0.5 or 0.1 and 0.25. In embodiments, this ratio may be calculated for the polymeric coating of the synthetic nanocarriers. In other embodiments, this ratio is calculated for the synthetic nanocarriers as a whole. The polymers coupled to the B cell antigen and the polymers not coupled to the B cell antigen may be the same type of polymer or may be different types of polymers. In one embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole. In another embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. Again, in a certain preferred embodiment, the polymer and other polymer both have a molecular weight of 5000 g/mole. As above the molecular weight may be the weight average molecular weight or it may be the number average molecular weight.

[0049] In yet another preferred embodiment, the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer not coupled to the B cell antigen, polymer coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen, or polymer not coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen is greater than 0.1, 0.25 or 0.5 and less than 1. Again, this ratio may be calculated based on the polymeric coating of the synthetic nanocarriers. In other embodiments, this ratio is calculated based on the synthetic nanocarriers as a whole. Again, the polymers coupled to the B cell antigen and the polymers not coupled to the B cell antigen may be the same type of polymer or may be different types of polymers. In one embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole. In another embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. Again, in a certain preferred embodiment, the polymer and other polymer both have a molecular weight of 5000 g/mole. The molecular weight may be a weight average molecular weight or it may be a number average molecular weight.

[0050] In one embodiment, any of the ratios referred to herein can be based on the polymeric coating across the population of synthetic nanocarriers. In another embodiment, the ratio is based on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

[0051] In another aspect, dosage forms and vaccines comprising any of the compositions provided herein are provided.

[0052] In still another aspect, any of the compositions may be administered to a subject in need thereof. The subject may have or be at risk of having cancer, an infection or infectious disease, a non-autoimmune or degenerative disease or an addiction.

[0053] The invention will now be described in more detail below.

B. DEFINITIONS

[0054] "Addiction antigens" are antigens associated with an addiction or addictive substance. Such antigens include those that can generate an antibody response against an addictive substance. Such antigens can comprise an addictive substance or a portion thereof.

[0055] "Adjuvant" means an agent that does not constitute a specific antigen, but boosts the strength and longevity of immune response to a concomitantly administered antigen. Such adjuvants may include, but are not limited to stimulators of pattern recognition receptors, such as Toll-like receptors, RIG-1 and NOD-like receptors (NLR), mineral salts, such as alum, alum combined with monphosphoryl lipid (MPL) A of Enterobacteria, such as *Escherihia coli, Salmonella minnesota, Salmonella typhimurium*, or *Shigella flexneri* or specifically with MPL® (AS04), MPL A of above-mentioned bacteria separately, saponins, such as QS-21, Quil-A, ISCOMs, ISCOMATRIX™, emulsions such as MF59™, Montanide® ISA 51 and ISA 720, AS02 (QS21+ squalene+MPL®), liposomes and liposomal formulations such as AS01, synthesized or specifically prepared microparticles and microcarriers such as bacteria-derived outer membrane vesicles (OMV) of *N. gonorrheae, Chlamydia trachomatis* and others, or chitosan particles, depot-forming agents, such as Pluronic® block co-polymers, specifically modified or prepared peptides, such as muramyl dipeptide, aminoalkyl glucosaminide 4-phosphates, such as RC529, or proteins, such as bacterial toxoids or toxin fragments.

[0056] In embodiments, adjuvants comprise agonists for pattern recognition receptors (PRR), including, but not limited to Toll-Like Receptors (TLRs), specifically TLRs 2, 3, 4, 5, 7, 8, 9 and/or combinations thereof. In other embodiments, adjuvants comprise agonists for Toll-Like Receptors 3, agonists for Toll-Like Receptors 7 and 8, or agonists for Toll-Like Receptor 9; preferably the recited adjuvants comprise imidazoquinolines; such as R848; adenine derivatives, such as those disclosed in U.S. Pat. No. 6,329,381 (Sumitomo Pharmaceutical Company), US Published Patent Application 2010/0075995 to Biggadike et al., or WO 2010/018132 to Campos et al.; immunostimulatory DNA; or immunostimulatory RNA. In specific embodiments, synthetic nanocarriers incorporate as adjuvants compounds that are agonists for toll-like receptors (TLRs) 7 & 8 ("TLR 7/8 agonists"). Of utility are the TLR 7/8 agonist compounds disclosed in U.S. Pat. No. 6,696,076 to Tomai et al., including but not limited to imidazoquinoline amines, imidazopyridine amines, 6,7-fused cycloalkylimidazopyridine amines, and 1,2-bridged imidazoquinoline amines. Preferred adjuvants comprise imiquimod and resiquimod (also known as R848). In specific

embodiments, an adjuvant may be an agonist for the DC surface molecule CD40. In certain embodiments, to stimulate immunity rather than tolerance, a synthetic nanocarrier incorporates an adjuvant that promotes DC maturation (needed for priming of naive T cells) and the production of cytokines, such as type I interferons, which promote antibody immune responses. In embodiments, adjuvants also may comprise immunostimulatory RNA molecules, such as but not limited to dsRNA, poly I:C or poly I:poly C12U (available as Ampligen®, both poly I:C and poly I:polyC12U being known as TLR3 stimulants), and/or those disclosed in F. Heil et al., "Species-Specific Recognition of Single-Stranded RNA via Toll-like Receptor 7 and 8" Science 303(5663), 1526-1529 (2004); J. Vollmer et al., "Immune modulation by chemically modified ribonucleosides and oligoribonucleotides" WO 2008033432 A2; A. Forsbach et al., "Immunostimulatory oligoribonucleotides containing specific sequence motif(s) and targeting the Toll-like receptor 8 pathway" WO 2007062107 A2; E. Uhlmann et al., "Modified oligoribonucleotide analogs with enhanced immunostimulatory activity" U.S. Pat. Appl. Publ. 2006241076; G. Lipford et al., "Immunostimulatory viral RNA oligonucleotides and use for treating cancer and infections" WO 2005097993 A2; G. Lipford et al, "Immunostimulatory G,U-containing oligoribonucleotides, compositions, and screening methods" WO 2003086280 A2. In some embodiments, an adjuvant may be a TLR-4 agonist, such as bacterial lipopolysacccharide (LPS), VSV-G, and/or HMGB-1. In some embodiments, adjuvants may comprise TLR-5 agonists, such as flagellin, or portions or derivatives thereof, including but not limited to those disclosed in U.S. Pat. Nos. 6,130,082, 6,585,980, and 7,192,725. In specific embodiments, synthetic nanocarriers incorporate a ligand for Toll-like receptor (TLR)-9, such as immunostimulatory DNA molecules comprising CpGs, which induce type I interferon secretion, and stimulate T and B cell activation leading to increased antibody production and cytotoxic T cell responses (Krieg et al., CpG motifs in bacterial DNA trigger direct B cell activation. Nature. 1995. 374: 546-549; Chu et al. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. J. Exp. Med. 1997. 186:1623-1631; Lipford et al. CpG-containing synthetic oligonucleotides promote B and cytotoxic T cell responses to protein antigen: a new class of vaccine adjuvants. Eur. J. Immunol. 1997. 27:2340-2344; Roman et al Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. Nat. Med. 1997. 3:849-854; Davis et al. CpG DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis B surface antigen. J. Immunol. 1998. 160:870-876; Lipford et al., Bacterial DNA as immune cell activator. Trends Microbiol. 1998. 6:496-500; U.S. Pat. No. 6,207,646 to Krieg et al.; U.S. Pat. No. 7,223, 398 to Tuck et al.; U.S. Pat. No. 7,250,403 to Van Nest et al.; or U.S. Pat. No. 7,566,703 to Krieg et al.

[0057] In some embodiments, adjuvants may be proinflammatory stimuli released from necrotic cells (e.g., urate crystals). In some embodiments, adjuvants may be activated components of the complement cascade (e.g., CD21, CD35, etc.). In some embodiments, adjuvants may be activated components of immune complexes. The adjuvants also include complement receptor agonists, such as a molecule that binds to CD21 or CD35. In some embodiments, the complement receptor agonist induces endogenous complement opsonization of the synthetic nanocarrier. In some embodiments, adjuvants are cytokines, which are small proteins or biological factors (in the range of 5 kD-20 kD) that are released by cells and have specific effects on cell-cell interaction, communication and behavior of other cells. In some embodiments, the cytokine receptor agonist is a small molecule, antibody, fusion protein, or aptamer.

[0058] In embodiments, at least a portion of the dose of adjuvant may be coupled to synthetic nanocarriers, preferably, all of the dose of adjuvant is coupled to synthetic nanocarriers. In other embodiments, at least a portion of the dose of the adjuvant is not coupled to the synthetic nanocarriers. In embodiments, the dose of adjuvant comprises two or more types of adjuvants or multiple adjuvants of the same type. For instance, and without limitation, adjuvants that act on different TLR receptors may be combined. As an example, in an embodiment a TLR 7/8 agonist may be combined with a TLR9 agonist. In another embodiment, a TLR 7/8 agonist may be combined with a TLR9 agonist. In yet another embodiment, a TLR9 agonist may be combined with a TLR9 agonist. In another embodiment, two TLR9 agonists may be combined.

[0059] "Administering" or "administration" means providing a material, such as a drug, to a subject in a manner that is pharmacologically useful.

[0060] "Amount effective" is any amount of a composition provided herein that produces one or more desired responses, such as one or more desired immune responses. This amount can be for in vitro or in vivo purposes. For in vivo purposes, the amount can be one that a clinician would believe may have a clinical benefit for a subject in need of a humoral immune response to a B cell antigen. Such subjects include those that have or are at risk of having cancer, an infection or infectious disease, a non-autoimmune or degenerative disease or an addiction.

[0061] Amounts effective include those that involve the production of an antibody response against a B cell antigen administered in one of the inventive compositions provided herein. A subject's antibody response can be monitored by routine methods. An amount that is effective to produce one or more desired immune responses can also be an amount of a composition provided herein that produces a desired therapeutic endpoint or a desired therapeutic result.

[0062] Amounts effective will depend, of course, on the particular subject being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reason.

[0063] In general, doses of the compositions of the invention can range from about 10 μg/kg to about 100,000 μg/kg. In some embodiments, the doses can range from about 0.1 mg/kg to about 100 mg/kg. In still other embodiments, the doses can range from about 0.1 mg/kg to about 25 mg/kg, about 25 mg/kg to about 50 mg/kg, about 50 mg/kg to about 75 mg/kg or about 75 mg/kg to about 100 mg/kg. Alternatively, the dose can be administered based on the number of

synthetic nanocarriers. For example, useful doses include greater than 10^6, 10^7, 10^8, 10^9 or 10^{10} synthetic nanocarriers per dose. Other examples of useful doses include from about 1×10^6 to about 1×10^{10}, about 1×10^7 to about 1×10^9 or about 1×10^8 to about 1×10^9 synthetic nanocarriers per dose.

[0064] "Antibody response" refers to the generation of antibodies specific for an antigen. An antibody response can target a desired B cell antigen (i.e., a desired antibody response) or to an off-target B cell antigen (i.e., an undesired antibody response). Preferably, the desired antibody responses are specific to the coupled B cell antigen of the compositions provided. Undesired antibody responses can interfere with desired antibody responses and include, for example, undesired antibody responses to the synthetic nanocarrier or a component thereof (e.g., a polymer, portion or unit thereof) of a synthetic nanocarrier. As a result, the compositions provided herein have been devised to include synthetic nanocarriers with an off-target response attenuating polymeric coating that elicits a desired antibody response to a coupled B cell antigen that is at least two-fold greater than an undesired antibody response, such as to the synthetic nanocarrier or component thereof. As provided elsewhere herein, the level of antibody response can be measured as a titer with an ELISA.

[0065] Methods for measuring an antibody response are known to those of ordinary skill in the art and are also exemplified below in the EXAMPLES. In particular, the antibody response can be quantitated, for example, as the number of antibodies, concentration of antibodies or titer. The values can be absolute or they can be relative. Assays for quantifying an antibody response include antibody capture assays, enzyme-linked immunosorbent assays (ELISAs), inhibition liquid phase absorption assays (ILPAAs), rocket immunoelectrophoresis (RIE) assays and line immunoelectrophoresis (LIE) assays. When a desired antibody response is compared to an undesired antibody response the same type of quantitative value (e.g., titer) and method of measurement (e.g., ELISA) is used to make the comparison.

[0066] An ELISA method for measuring an antibody titer, for example, may consist of the following steps (i) preparing an ELISA-plate coating material such that the antibody target of interest is coupled to a substrate polymer or other suitable material (ii) preparing the coating material in an aqueous solution (such as PBS) and delivering the coating material solution to the wells of a multiwell plate for overnight deposition of the coating onto the multiwell plate (iii) thoroughly washing the multiwell plate with wash buffer (such as 0.05% Tween-20 in PBS) to remove excess coating material (iv) blocking the plate for nonspecific binding by applying a diluent solution (such as 10% fetal bovine serum in PBS), (v) washing the blocking/diluent solution from the plate with wash buffer (vi) diluting the serum sample(s) containing antibodies and appropriate standards (positive controls) with diluent as required to obtain a concentration that suitably saturates the ELISA reponse (vii) serially diluting the plasma samples on the multiwell plate such to cover a range of concentrations suitable for generating an ELISA response curve (viii) incubating the plate to provide for antibody-target binding (ix) washing the plate with wash buffer to remove antibodies not bound to antigen (x) adding an appropriate concentration of a secondary detection antibody in same diluent such as a biotin-coupled detection antibody capable of binding the primary antibody (xi) incubating the plate with the applied detection antibody, followed by washing with wash buffer (xii) adding an enzyme such as streptavidin-HRP (horse radish peroxidase) that will bind to biotin found on biotinylated antibodies and incubating (xiii) washing the multiwell plate (xiv) adding substrate(s) (such as TMB solution) to the plate (xv) applying a stop solution (such as 2N sulfuric acid) when color development is complete (xvi) reading optical density of the plate wells at a specific wavelength for the substrate (450 nm with subtraction of readings at 570 nm) (xvi) applying a suitable multiparameter curve fit to the data and defining half-maximal effective concentration (EC50) as the concentration on the curve at which half the maximum OD value for the plate standards is achieved.

[0067] "Antigen" means a B cell antigen or T cell antigen. In embodiments, antigens are coupled to the synthetic nanocarriers. In other embodiments, antigens are not coupled to the synthetic nanocarriers. "Type(s) of antigens" means molecules that share the same, or substantially the same, antigenic characteristics.

[0068] An "at risk" subject is one in which a health practitioner believes has a chance of having a disease or condition as provided herein.

[0069] "Average", as used herein, refers to the arithmetic mean unless otherwise noted.

[0070] "Average number of polymers" is an absolute or relative value for the number of polymers averaged across a population of synthetic nanocarriers. Methods for determining the average number of polymers are known to those of ordinary skill in the art. For example, the average number of polymers in a formulated population may be obtained by determining the total weight of the polymer in the population and dividing by the number-averaged molecular weight. When the ratio of polymers as provided herein is calculated for a particular synthetic nanocarrier population the same type of value (absolute or relative) measured according to the same type of assay is used.

[0071] "B cell antigen" means any antigen that is recognized by or triggers an immune response in a B cell (e.g., an antigen that is specifically recognized by a B cell or a receptor thereon). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. B cell antigens include, but are not limited to proteins, peptides, small molecules, oligosaccharides, and carbohydrates. In some embodiments, the B cell antigen comprises a non-protein antigen (i.e., not a protein or peptide antigen). In some embodiments, the B cell antigen comprises a carbohydrate associated with an infectious agent. In some embodiments, the B cell antigen comprises a glycoprotein or glycopeptide associated with an infectious agent. The infectious agent can be a bacterium, virus, fungus, protozoan, or parasite. In some embodiments, the B cell antigen comprises a poorly immunogenic antigen. In some embodiments, the B cell antigen comprises an abused substance or a portion thereof. In some embodiments, the B cell antigen comprises an addictive substance or a portion thereof. Addictive substances include, but are not limited to, nicotine, a narcotic, a cough suppressant, a tranquilizer, and a sedative. In some embodiments, the B cell antigen comprises a toxin, such as a toxin from a chemical weapon or natural sources. The B cell antigen may also comprise a hazardous environmental agent. In some embodiments, the B cell antigen comprises a self antigen. In other embodiments, the B cell antigen comprises an alloantigen, an allergen, a contact sensitizer, a degenerative disease antigen, a hapten, an infectious disease antigen, a cancer antigen, an

atopic disease antigen, an autoimmune disease antigen, a non-autoimmune disease antigen, an addictive substance, a xenoantigen, or a metabolic disease enzyme or enzymatic product thereof.

[0072] Generally, as used herein and unless otherwise noted, "B cell antigen" of the compositions provided refers to a B cell antigen to which a target antibody response is desired and not to an antigen to which an antibody response is not desired (e.g., against the carrier or synthetic component thereof (e.g., a polymer of the synthetic nanocarrier)).

[0073] "Cancer antigens" are antigens associated with a cancer or cancerous tumor. Such antigens can generate an antibody response against a cancer or tumor cell. Such antigens can comprise an antigen that is expressed in or on cancer or tumor cells but not in or on normal or healthy cells. Such antigens can also comprise an antigen that is expressed in or on cancer or tumor cells and on normal or healthy cells but is expressed in or on cancer or tumor cells at a greater level than on normal or healthy cells. Preferably, the use of a cancer antigen in such an embodiment will not lead to a substantial or detrimental immune response against normal or healthy cells or will lead to a beneficial immune response against the cancer or tumor cells that outweighs any immune response against normal or healthy cells.

[0074] "Couple" or "Coupled" or "Couples" (and the like) means to chemically associate one entity (for example a moiety) with another. In some embodiments, the coupling is covalent, meaning that the coupling occurs in the context of the presence of a covalent bond between the two entities. In non-covalent embodiments, the non-covalent coupling is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, TT stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. In embodiments, encapsulation is a form of coupling.

[0075] "Dosage form" means a pharmacologically and/or immunologically active material in a medium, carrier, vehicle, or device suitable for administration to a subject.

[0076] "Encapsulate" means to enclose at least a portion of a substance within a synthetic nanocarrier. In some embodiments, a substance is enclosed completely within a synthetic nanocarrier. In other embodiments, most or all of a substance that is encapsulated is not exposed to the local environment external to the synthetic nanocarrier. In other embodiments, no more than 50%, 40%, 30%, 20%, 10% or 5% (weight/weight) of the substance is exposed to the local environment. Encapsulation is distinct from absorption, which places most or all of a substance on a surface of a synthetic nanocarrier, and leaves the substance exposed to the local environment external to the synthetic nanocarrier. In some embodiments, the polymeric coating provided herein encapsulates one or more or all of the other substances of a synthetic nanocarrier provided. In one embodiment, these other substances do not include desired B cell antigen coupled to the polymeric coating at the surface of the synthetic nanocarrier.

[0077] "Humoral response" means any immune response that results in the production or stimulation of B cells and/or the production of antibodies. Preferably, the humoral immune response is specific to an antigen comprised within an inventive composition or administered during the practice of an inventive method. Methods for assessing whether a humoral response is induced are known to those of ordinary skill in the art. Examples of such methods are provided below in the Examples.

[0078] An "infection" or "infectious disease" is any condition or disease caused by a microorganism, pathogen or other agent, such as a bacterium, fungus, prion or virus. "An infection or infectious disease antigen" is an antigen associated with an infection or infectious disease. Such antigens include antigens that can be used to generate an antibody response against a pathogen or other infectious agent, or component thereof, or that can generate an antibody response against infected cells.

[0079] "Maximum dimension of a synthetic nanocarrier" means the largest dimension of a nanocarrier measured along any axis of the synthetic nanocarrier. "Minimum dimension of a synthetic nanocarrier" means the smallest dimension of a synthetic nanocarrier measured along any axis of the synthetic nanocarrier. For example, for a spheroidal synthetic nanocarrier, the maximum and minimum dimension of a synthetic nanocarrier would be substantially identical, and would be the size of its diameter. Similarly, for a cuboidal synthetic nanocarrier, the minimum dimension of a synthetic nanocarrier would be the smallest of its height, width or length, while the maximum dimension of a synthetic nanocarrier would be the largest of its height, width or length. In an embodiment, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm. In an embodiment, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or less than 5 μm. Preferably, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is greater than 110 nm, more preferably greater than 120 nm, more preferably greater than 130 nm, and more preferably still greater than 150 nm. Aspects ratios of the maximum and minimum dimensions of inventive synthetic nanocarriers may vary depending on the embodiment. For instance, aspect ratios of the maximum to minimum dimensions of the synthetic nanocarriers may vary from 1:1 to 1,000, 000:1, preferably from 1:1 to 100, 000:1, more preferably from 1:1 to 10,000:1, more preferably from 1:1 to 1000:1, still more preferably from 1:1 to 100:1, and yet more preferably from 1:1 to 10:1. Preferably, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample is equal to or less than 3 μm, more preferably equal to or less than 2 μm, more preferably equal to or less than 1 μm, more preferably equal to or less than 800 nm, more preferably equal to or less than 600 nm, and more preferably still equal to or less than 500 nm. In preferred embodiments, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm, more preferably equal to or greater than 120 nm, more preferably equal to or greater than 130 nm, more preferably equal to or greater than 140 nm, and more preferably still equal to or greater than 150 nm. Measurement of synthetic nanocarrier dimensions (e.g., diameter) is obtained by suspending the

synthetic nanocarriers in a liquid (usually aqueous) media and using dynamic light scattering (DLS) (e.g. using a Brookhaven ZetaPALS instrument). For example, a suspension of synthetic nanocarriers can be diluted from an aqueous buffer into purified water to achieve a final synthetic nanocarrier suspension concentration of approximately 0.01 to 0.1 mg/mL. The diluted suspension may be prepared directly inside, or transferred to, a suitable cuvette for DLS analysis. The cuvette may then be placed in the DLS, allowed to equilibrate to the controlled temperature, and then scanned for sufficient time to acquire a stable and reproducible distribution based on appropriate inputs for viscosity of the medium and refractive indicies of the sample. The effective diameter, or mean of the distribution, is then reported. "Dimension" or "size" or "diameter" of synthetic nanocarriers means the mean of a particle size distribution obtained using dynamic light scattering.

[0080] "Non-autoimmune or degenerative antigens" are antigens associated with non-autoimmune or degenerative diseases or conditions. Such antigens can result in an antibody response that can be indicative of and/or present when the non-autoimmune or degenerative disease or condition occurs or is present in a subject. Such antigens can also be used to generate an antibody response, the generation of which may be beneficial in the treatment or prevention of the disease or condition or one or more symptoms thereof.

[0081] "Off-target response attenuating polymeric coating" refers to a composition comprising one or more polymers present at at least a portion of the surface of a synthetic nanocarrier and that when the synthetic nanocarrier is coupled to a B cell antigen, the synthetic nanocarrier, or population thereof, generates an antibody response against the B cell antigen that is at least two-fold greater than an off-target (or undesired) antibody response, such as against the synthetic nanocarrier or component thereof (such as a polymer of the coating). In one embodiment, the antibody response against the B cell antigen and the off-target antibody response are of the same type, such as both an IgG or IgA antibody response. In another embodiment, the synthetic nanocarrier, or population thereof, generates an antibody response that is at least 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65-, 70-, 75-, 80-, 85-, 90-, 95- or 100-fold greater. In another embodiment, the response is measured as an antibody titer (e.g., IgG or IgA EC50) with an ELISA. In another embodiment, the coating may be present throughout the surface of a synthetic nanocarrier.

[0082] The coating may comprise a number of polymers of the same type or it may comprise a number of polymers of two or more different types. The polymers of the coating may comprise PEG, a polyethyloxazoline, a polyamino acid, polycarbonate, hydrophilic polyacetal, hydrophilic polyketal, polysaccharide, polypropylene or polyethyleneimine, or some combination thereof. The coating may comprise a number of the same type of the aforementioned polymers or may comprise a number of two or more types of the aforementioned polymers. In one embodiment, the coating comprises a number of polymers that comprise one or more of the aforementioned types of polymers, and it is the antibody response to one or more of these aforementioned types of polymers that is at least two-fold less than the antibody response to the target B cell antigen. In one embodiment, the antibody response is at least 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65-, 70-, 75-, 80-, 85-, 90-, 95- or 100-fold less. In another embodiment, the coating comprises polymers comprising PEG, and it is the antibody response to PEG that is at least two-fold less than the antibody response to the target B cell antigen. In a further embodiment, the antibody response to PEG is at least 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65-, 70-, 75-, 80-, 85-, 90-, 95- or 100-fold less. Again, the response may be measured as an antibody titer (e.g., IgG or IgA EC50) with an ELISA, and/or both responses are of the same type (e.g., both IgG or IgA antibody responses).

[0083] Preferably, in one embodiment, the coating comprises a polymer (e.g., one of the aforementioned polymers) with a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole. In another embodiment, the polymer has a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. The B cell antigen may be coupled to this polymer or to another polymer of the coating. The B cell antigen may also be coupled to another portion of the synthetic nanocarriers such as to the surface of the synthetic nanocarriers but not to the coating. In another embodiment, wherein the B cell antigen is coupled to another polymer of the coating, the other polymer also has a molecular weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole. In another embodiment, the other polymer has a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. In a certain preferred embodiment, the polymer and other polymer of the coating both have a molecular weight of 5000 g/mole. The molecular weight may be a weight average molecular weight or a number average molecular weight.

[0084] In another preferred embodiment, the ratio of the average number of polymers coupled to the B cell antigen of the coating across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen of the coating across the population of synthetic nanocarriers, the ratio of the average number of polymers coupled to the B cell antigen of the coating across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen of the coating across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen of the coating across the population of synthetic nanocarriers, or the ratio of the average number of polymers not coupled to the B cell antigen of the coating across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen of the coating across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen of the coating across the population of synthetic nanocarriers is between 0.001 and 1, 0.01 and 1, 0.1 and 1, 0.25 and 1, 0.5 and 1 or 0.75 and 1 or as elsewhere provided. In yet another preferred embodiment, the ratio by weight is 0.1, 0.25 or 0.5. In one embodiment, this ratio is calculated based on the polymeric coatings of the synthetic nanocarriers. In another embodiment, this ratio is calculated based on the synthetic nanocarriers as a whole. The polymers coupled to the B cell antigen and the polymers not coupled to the B cell antigen may be the same type of polymer or may be different types of polymers. In one embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular

weight of greater than 2000 g/mole, 3000 g/mole, 4000 g/mole or 5000 g/mole. In another embodiment, the polymers coupled to the B cell antigen and/or the polymers not coupled to the B cell antigen have a molecular weight of between 2000-5000 g/mole, between 2500-5000 g/mole, between 3000-5000 g/mole, between 3500-5000 g/mole or between 4000-5000 g/mole. Again, in a certain preferred embodiment, the polymer and other polymer both have a molecular weight of 5000 g/mole. The molecular weight may be the weight average molecular weight or the number average molecular weight.

[0085] "Off-target antibody response" is any undesired antibody response as provided herein. Generally, the off-target antibody response is an antibody response not specific to the B cell antigen coupled to the synthetic nanocarriers to which an antibody response is desired. In some embodiments, if the intended antibody response is IgG or IgA, it is desirable that this desired antibody response be at least two-fold greater than the off-target response by that same class. In some embodiments, an off-target IgM response that is of similar or greater magnitude than a desired IgG or IgA response may occur. Generally, IgM tends to be a transient low-affinity response whereas IgG is a longer-lasting higher-affinity response.

[0086] In one embodiment, the off-target antibody response is an antibody response against the synthetic nanocarrier or component thereof, such as a polymer (or portion thereof), such as of the coating.

[0087] "Pharmaceutically acceptable excipient" means a pharmacologically inactive material used together with the recited synthetic nanocarriers to formulate the inventive compositions.

[0088] Pharmaceutically acceptable excipients comprise a variety of materials known in the art, including but not limited to saccharides (such as glucose, lactose, and the like), preservatives such as antimicrobial agents, reconstitution aids, colorants, saline (such as phosphate buffered saline), and buffers.

[0089] "Ratio by weight averaged across the population of synthetic nanocarriers" refers to the ratio of absolute or relative values for two weights averaged across a population of synthetic nanocarriers. When the ratio of the weight of polymers is calculated for a particular synthetic nanocarrier population the same type of value (absolute or relative) measured according to the same type of assay is used. Methods for determining the weight of a certain type of polymer in synthetic nanocarriers are known to those of ordinary skill in the art. Examples of methods are also provided elsewhere herein. Alternatively, well-described polymers, such as those with information provided by a manufacturer can be formulated at a certain ratio.

[0090] "Same type of polymer" means polymers that share the same, or substantially the same, chemical structure. Polymers that are the same type of polymers may have the same or different molecular weights. In a preferred embodiment, polymers that are the same type of polymer also have the same molecular weight.

[0091] "Subject" means animals, including warm blooded mammals such as humans and primates; avians; domestic household or farm animals such as cats, dogs, sheep, goats, cattle, horses and pigs; laboratory animals such as mice, rats and guinea pigs; fish; reptiles; zoo and wild animals; and the like.

[0092] "Synthetic nanocarrier(s)" means a discrete object that is not found in nature, and that possesses at least one dimension that is less than or equal to 5 microns in size. Albumin nanoparticles are generally included as synthetic nanocarriers, however in certain embodiments the synthetic nanocarriers do not comprise albumin nanoparticles. In embodiments, synthetic nanocarriers do not comprise chitosan. In certain other embodiments, the synthetic nanocarriers do not comprise chitosan. In other embodiments, inventive synthetic nanocarriers are not lipid-based nanoparticles. In further embodiments, inventive synthetic nanocarriers do not comprise a phospholipid.

[0093] A synthetic nanocarrier can be, but is not limited to, one or a plurality of lipid-based nanoparticles (also referred to herein as lipid nanoparticles, i.e., nanoparticles where the majority of the material that makes up their structure are lipids), polymeric nanoparticles, metallic nanoparticles, surfactant-based emulsions, dendrimers, buckyballs, nanowires, virus-like particles (i.e., particles that are primarily made up of viral structural proteins but that are not infectious or have low infectivity), peptide or protein-based particles (also referred to herein as protein particles, i.e., particles where the majority of the material that makes up their structure are peptides or proteins) (such as albumin nanoparticles) and/or nanoparticles that are developed using a combination of nanomaterials such as lipid-polymer nanoparticles. Synthetic nanocarriers may be a variety of different shapes, including but not limited to spheroidal, cuboidal, pyramidal, oblong, cylindrical, toroidal, and the like. Synthetic nanocarriers according to the invention comprise one or more surfaces. Exemplary synthetic nanocarriers that can be adapted for use in the practice of the present invention comprise: (1) the biodegradable nanoparticles disclosed in U.S. Pat. No. 5,543,158 to Gref et al., (2) the polymeric nanoparticles of Published US Patent Application 20060002852 to Saltzman et al., (3) the lithographically constructed nanoparticles of Published US Patent Application 20090028910 to DeSimone et al., (4) the disclosure of WO 2009/051837 to von Andrian et al., (5) the nanoparticles disclosed in Published US Patent Application 2008/0145441 to Penades et al., (6) the protein nanoparticles disclosed in Published US Patent Application 20090226525 to de los Rios et al., (7) the virus-like particles disclosed in published US Patent Application 20060222652 to Sebbel et al., (8) the nucleic acid coupled virus-like particles disclosed in published US Patent Application 20060251677 to Bachmann et al., (9) the virus-like particles disclosed in WO2010047839A1 or WO2009106999A2, (10) the nanoprecipitated nanoparticles disclosed in P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010) or (11) apoptotic cells, apoptotic bodies or the synthetic or semisynthetic mimics disclosed in U.S. Publication 2002/0086049. In embodiments, synthetic nanocarriers may possess an aspect ratio greater than 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

[0094] Synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface with hydroxyl groups that activate complement or alternatively comprise a surface that consists essentially of moieties that are not hydroxyl groups that activate complement. In a preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably

equal to or less than 100 nm, do not comprise a surface that substantially activates complement or alternatively comprise a surface that consists essentially of moieties that do not substantially activate complement. In a more preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that activates complement or alternatively comprise a surface that consists essentially of moieties that do not activate complement. In embodiments, synthetic nanocarriers exclude virus-like particles. In embodiments, when synthetic nanocarriers comprise virus-like particles, the virus-like particles comprise non-natural adjuvant (meaning that the VLPs comprise an adjuvant other than naturally occurring RNA generated during the production of the VLPs). In embodiments, synthetic nanocarriers may possess an aspect ratio greater than 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

[0095] "T cell antigen" means any antigen that is recognized by and triggers an immune response in a T cell (e.g., an antigen that is specifically recognized by a T cell receptor on a T cell or an NKT cell via presentation of the antigen or portion thereof bound to a Class I or Class II major histocompatibility complex molecule (MHC), or bound to a CD1 complex). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. T cell antigens generally are proteins or peptides. T cell antigens may be an antigen that stimulates a CD8+ T cell response, a CD4+ T cell response, or both. The nanocarriers, therefore, in some embodiments can effectively stimulate both types of responses.

[0096] In some embodiments the T cell antigen is a T helper cell antigen (i.e. one that can generate an enhanced response to a B cell antigen, preferably an unrelated B cell antigen, through stimulation of T cell help). In embodiments, a T helper cell antigen may comprise one or more peptides obtained or derived from tetanus toxoid, Epstein-Barr virus, influenza virus, respiratory syncytial virus, measles virus, mumps virus, rubella virus, cytomegalovirus, adenovirus, diphtheria toxoid, or a PADRE peptide (known from the work of Sette et al. U.S. Pat. No. 7,202,351). In other embodiments, a T helper cell antigen may comprise one or more lipids, or glycolipids, including but not limited to: α-galactosylceramide (α-GalCer), α-linked glycosphingolipids (from *Sphingomonas* spp.), galactosyl diacylglycerols (from *Borrelia burgdorferi*), lypophosphoglycan (from *Leishmania donovani*), and phosphatidylinositol tetramannoside (PIM4) (from *Mycobacterium leprae*). For additional lipids and/or glycolipids useful as a T helper cell antigen, see V. Cerundolo et al., "Harnessing invariant NKT cells in vaccination strategies." Nature Rev Immun, 9:28-38 (2009). In embodiments, CD4+ T-cell antigens may be derivatives of a CD4+ T-cell antigen that is obtained from a source, such as a natural source. In such embodiments, CD4+ T-cell antigen sequences, such as those peptides that bind to MHC II, may have at least 70%, 80%, 90%, or 95% identity to the antigen obtained from the source. In embodiments, the T cell antigen, preferably a T helper cell antigen, may be coupled to, or uncoupled from, a synthetic nanocarrier. In some embodiments, the T cell antigen is encapsulated in the synthetic nanocarriers of the compositions.

[0097] "Vaccine" means a composition of matter that improves the immune response to a particular pathogen or disease. A vaccine typically contains factors that stimulate a subject's immune system to recognize a specific antigen as foreign and eliminate it from the subject's body. A vaccine also establishes an immunologic 'memory' so the antigen will be quickly recognized and responded to if a person is re-challenged. Vaccines can be prophylactic (for example to prevent future infection by any pathogen), or therapeutic (for example a vaccine against a tumor specific antigen for the treatment of cancer). In embodiments, a vaccine may comprise dosage forms according to the invention.

[0098] "Weight", as used herein, refers to mass unless otherwise noted. When a molecular weight of a polymer is measured, it can be measured as the weight average molecular weight or a number average molecular weight. "Weight average molecular weight" for the polymers of the compositions provided herein is calculated by the following formula:

$$\overline{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}, \qquad \text{Formula 1}$$

where Ni is the number of molecules of molecular weight Mi. The weight average molecular weight can be determined by a variety of methods including light scattering, small angle neutron scattering (SANS), X-ray scattering, Nuclear Magnetic Resonance (NMR) and sedimentation velocity. An example of an alternative for weight average molecular weight is to perform gel permeation chromatography using suitable traceable-weight standards to establish a retention-time versus weight curve, and calculating the mean weight-averaged molecular weight of a sample polymer from the mean of the integrated sample peak as compared to the calibration curve. The "number average molecular weight" can be determined by NMR. For example, number average molecular weight can be determined by proton NMR wherein the ratio of the polymer repeating units to the end group is established and then multiplied by theoretical repeating unit molecular weight. Alternatively, in the case of a titratable (e.g., acid or base) end group polymer, a known weight concentration may be established and then titrated in the presense of an indicator dye with an appropriate neutralizing agent of known molar concentration to provide moles of end group per mass of polymer.

C. INVENTIVE COMPOSITIONS

[0099] Provided herein are compositions comprising synthetic nanocarriers that provide optimized target antibody generation to a B cell antigen relative to off-target antibody generation. These synthetic nanocarriers comprise a B cell antigen and an off-target response attenuating polymeric coating. It has been found that optimized target antibody generation relative to off-target antibody generation results when a polymeric coating comprises certain B cell antigen content and/or polymer molecular weights and compositions.

[0100] It has been found that coatings that provide optimized B cell antigen response relative to off-target antibody response may comprise polymers with certain molecular weights (as weight average or number average) with polymers with greater molecular weights having better effect. The coating may comprise one type of polymer (with an aforementioned molecular weight) but may also comprise one or more other types of polymers. The one or more other types of

polymers may also have the aforementioned molecular weights. The one or more types of polymers of the coating may be in the form of a polymeric matrix.

[0101] The target B cell antigen may be coupled to one of the types of polymers of the coating or to more than one of the types of polymers of the coating. In another embodiment, the target B cell antigen is coupled to the polymer of the coating for which an attenuated antibody response is desired. When a target B cell antigen is coupled to one or more of the types of polymers of the coating, the target B cell antigen is coupled to all or less than all of the polymer molecules of the one or more types of polymers of the coating. The target B cell antigen may also be coupled to another component of the synthetic nanocarriers, such as the surface of the synthetic nanocarrier, but not to the coating. The target B cell antigen can be coupled, in some embodiments, by any means known in the art. In one embodiment, the target B cell antigen is coupled via a bond or linker.

[0102] It has also been found that the amount of antigen coupled to the off-target response attenuating polymeric coating can also provide optimized B cell antigen response relative to off-target antibody response. It has been found that an increased amount of antigen present in the coating of the synthetic nanocarrier provides attenuated off-target antibody response relative to target antibody response. In another preferred embodiment, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, or the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers may be between 0.001 and 1, 0.01 and 1, 0.1 and 1, 0.25 and 1, 0.5 and 1 or 0.75 and 1. The ratio can be calculated based on an assessment of the polymeric coating across the population of synthetic nanocarriers or of the synthetic nanocarriers as a whole across the population of synthetic nanocarriers. The polymers coupled to the B cell antigen and the polymers not coupled to the B cell antigen of the coating may be the same type of polymer or may be different types of polymers. These polymers may also have the molecular weights provided above in some embodiments.

[0103] As mentioned above, the polymers of the coating may comprise a number of polymers of the same type or it may comprise a number of polymers of two or more different types. The polymers of the coating may comprise PEG, a polyethyloxazoline, a polyamino acid, polycarbonate, hydrophilic polyacetal, hydrophilic polyketal, polypropylene, polysaccharide or polyethyleneimine, or some combination thereof. In preferred embodiments, the polymers of the coating comprise PEG.

[0104] The off-target response attenuating polymeric coating may be a coating on a number of different types of synthetic nanocarriers. Accordingly, a wide variety of synthetic nanocarriers can be used according to the invention. In some embodiments, synthetic nanocarriers are spheres or spheroids. In some embodiments, synthetic nanocarriers are flat or plate-shaped. In some embodiments, synthetic nanocarriers are cubes or cubic. In some embodiments, synthetic nanocarriers are ovals or ellipses. In some embodiments, synthetic nanocarriers are cylinders, cones, or pyramids.

[0105] In some embodiments, it is desirable to use a population of synthetic nanocarriers that is relatively uniform in terms of size, shape, and/or composition so that each synthetic nanocarrier has similar properties. For example, at least 80%, at least 90%, or at least 95% of the synthetic nanocarriers, based on the total number of synthetic nanocarriers, may have a minimum dimension or maximum dimension that falls within 5%, 10%, or 20% of the average diameter or average dimension of the synthetic nanocarriers. In some embodiments, a population of synthetic nanocarriers may be heterogeneous with respect to size, shape, and/or composition.

[0106] Synthetic nanocarriers can be solid or hollow and can comprise one or more layers. In some embodiments, each layer has a unique composition and unique properties relative to the other layer(s). To give but one example, synthetic nanocarriers may have a core/shell structure, wherein the core is one layer (e.g. a polymeric core) and the shell is a second layer (e.g. a lipid bilayer or monolayer). Synthetic nanocarriers may comprise a plurality of different layers.

[0107] In some embodiments, synthetic nanocarriers may optionally comprise one or more lipids. In some embodiments, a synthetic nanocarrier may comprise a liposome. In some embodiments, a synthetic nanocarrier may comprise a lipid bilayer. In some embodiments, a synthetic nanocarrier may comprise a lipid monolayer. In some embodiments, a synthetic nanocarrier may comprise a micelle. In some embodiments, a synthetic nanocarrier may comprise a core comprising a polymeric matrix surrounded by a lipid layer (e.g., lipid bilayer, lipid monolayer, etc.). In some embodiments, a synthetic nanocarrier may comprise a non-polymeric core (e.g., metal particle, quantum dot, ceramic particle, bone particle, viral particle, proteins, nucleic acids, carbohydrates, etc.) surrounded by a lipid layer (e.g., lipid bilayer, lipid monolayer, etc.).

[0108] In some embodiments, synthetic nanocarriers can comprise one or more other polymers. In some embodiments, various elements of the synthetic nanocarriers can be coupled with such polymers. Such other polymers may form a polymeric matrix, and the components of the synthetic nanocarriers may be covalently associated with the polymeric matrix. In some embodiments, covalent association is mediated by a linker. In some embodiments, a component may be noncovalently associated with the polymeric matrix. For example, in some embodiments, a component may be encapsulated within, surrounded by, and/or dispersed throughout a polymeric matrix. Alternatively or additionally, a component can be associated with a polymeric matrix by hydrophobic interactions, charge interactions, van der Waals forces, etc. In some embodiments, where the polymers of the coating form a polymeric matrix, components can also be coupled thereto by these aforementioned methods.

[0109] A wide variety of polymers and methods for forming polymeric matrices therefrom are known conventionally. In general, a polymeric matrix comprises one or more polymers. Polymers may be natural or unnatural (synthetic) polymers. Polymers may be homopolymers or copolymers comprising two or more monomers. In terms of sequence,

copolymers may be random, block, or comprise a combination of random and block sequences. Typically, polymers in accordance with the present invention are organic polymers.

[0110] Examples of polymers suitable for use in the synthetic nanocarriers, as part of the coating or other portion of the synthetic nanocarriers, include, but are not limited to polyethylenes, polycarbonates (e.g. poly(1,3-dioxan-2one)), polyanhydrides (e.g. poly(sebacic anhydride)), polypropylfumerates, polyamides (e.g. polycaprolactam), polyacetals, polyethers, polyesters (e.g., polylactide, polyglycolide, polylactide-co-glycolide, polycaprolactone, polyhydroxyacid (e.g. poly(β-hydroxyalkanoate))), poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polyureas, polystyrenes, and polyamines, polylysine, polylysine-PEG copolymers, and poly(ethyleneimine), poly(ethylene imine)-PEG copolymers. In some embodiments, polymers in accordance with the present invention include polymers which have been approved for use in humans by the U.S. Food and Drug Administration (FDA) under 21 C.F.R. §177.2600, including but not limited to polyesters (e.g., polylactic acid, poly(lactic-co-glycolic acid), polycaprolactone, polyvalerolactone, poly(1,3-dioxan-2one)); polyanhydrides (e.g., poly (sebacic anhydride)); polyethers (e.g., polyethylene glycol); polyurethanes; polymethacrylates; polyacrylates; and polycyanoacrylates.

[0111] In some embodiments, polymers can be hydrophilic. For example, polymers may comprise anionic groups (e.g., phosphate group, sulphate group, carboxylate group); cationic groups (e.g., quaternary amine group); or polar groups (e.g., hydroxyl group, thiol group, amine group). In some embodiments, a synthetic nanocarrier comprising a hydrophilic polymeric matrix generates a hydrophilic environment within the synthetic nanocarrier. In some embodiments, polymers can be hydrophobic. In some embodiments, a synthetic nanocarrier comprising a hydrophobic polymeric matrix generates a hydrophobic environment within the synthetic nanocarrier. Selection of the hydrophilicity or hydrophobicity of the polymer may have an impact on the nature of materials that are incorporated (e.g. coupled) within the synthetic nanocarrier.

[0112] In some embodiments, polymers may be modified with one or more moieties and/or functional groups. A variety of moieties or functional groups can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from polysaccharides (Papisov, 2001, ACS Symposium Series, 786:301). Certain embodiments may be made using the general teachings of U.S. Pat. No. 5,543,158 to Gref et al., or WO publication WO2009/051837 by Von Andrian et al.

[0113] In some embodiments, polymers may be modified with a lipid or fatty acid group. In some embodiments, a fatty acid group may be one or more of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, or lignoceric acid. In some embodiments, a fatty acid group may be one or more of palmitoleic, oleic, vaccenic, linoleic, alpha-linoleic, gamma-linoleic, arachidonic, gadoleic, arachidonic, eicosapentaenoic, docosahexaenoic, or erucic acid.

[0114] In some embodiments, polymers may be polyesters, including copolymers comprising lactic acid and glycolic acid units, such as poly(lactic acid-co-glycolic acid) and poly (lactide-co-glycolide), collectively referred to herein as "PLGA"; and homopolymers comprising glycolic acid units, referred to herein as "PGA," and lactic acid units, such as poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, poly-L-lactide, poly-D-lactide, and poly-D,L-lactide, collectively referred to herein as "PLA." In some embodiments, exemplary polyesters include, for example, polyhydroxyacids; PEG copolymers and copolymers of lactide and glycolide (e.g., PLA-PEG copolymers, PGA-PEG copolymers, PLGA-PEG copolymers, and derivatives thereof. In some embodiments, polyesters include, for example, poly(caprolactone), poly(caprolactone)-PEG copolymers, poly(L-lactide-co-L-lysine), poly(serine ester), poly(4-hydroxy-L-proline ester), poly[α-(4-aminobutyl)-L-glycolic acid], and derivatives thereof.

[0115] In some embodiments, a polymer may be PLGA. PLGA is a biocompatible and biodegradable co-polymer of lactic acid and glycolic acid, and various forms of PLGA are characterized by the ratio of lactic acid:glycolic acid. Lactic acid can be L-lactic acid, D-lactic acid, or D,L-lactic acid. The degradation rate of PLGA can be adjusted by altering the lactic acid:glycolic acid ratio. In some embodiments, PLGA to be used in accordance with the present invention is characterized by a lactic acid:glycolic acid ratio of approximately 85:15, approximately 75:25, approximately 60:40, approximately 50:50, approximately 40:60, approximately 25:75, or approximately 15:85.

[0116] In some embodiments, polymers may be one or more acrylic polymers. In certain embodiments, acrylic polymers include, for example, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), methacrylic acid alkylamide copolymer, poly(methyl methacrylate), poly(methacrylic acid anhydride), methyl methacrylate, polymethacrylate, poly(methyl methacrylate) copolymer, polyacrylamide, aminoalkyl methacrylate copolymer, glycidyl methacrylate copolymers, polycyanoacrylates, and combinations comprising one or more of the foregoing polymers. The acrylic polymer may comprise fully-polymerized copolymers of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups.

[0117] In some embodiments, polymers can be cationic polymers. In general, cationic polymers are able to condense and/or protect negatively charged strands of nucleic acids (e.g. DNA, or derivatives thereof). Amine-containing polymers such as poly(lysine) (Zauner et al., 1998, Adv. Drug Del. Rev., 30:97; and Kabanov et al., 1995, Bioconjugate Chem., 6:7), poly(ethylene imine) (PEI; Boussif et al., 1995, Proc. Natl. Acad. Sci., USA, 1995, 92:7297), and poly(amidoamine) dendrimers (Kukowska-Latallo et al., 1996, Proc. Natl. Acad. Sci., USA, 93:4897; Tang et al., 1996, Bioconjugate Chem., 7:703; and Haensler et al., 1993, Bioconjugate Chem., 4:372) are positively-charged at physiological pH, form ion pairs with nucleic acids, and mediate transfection in a variety of cell lines. In embodiments, the inventive synthetic nanocarriers may not comprise (or may exclude) cationic polymers.

[0118] In some embodiments, polymers can be degradable polyesters bearing cationic side chains (Putnam et al., 1999, Macromolecules, 32:3658; Barrera et al., 1993, J. Am. Chem. Soc., 115:11010; Kwon et al., 1989, Macromolecules, 22:3250; Lim et al., 1999, J. Am. Chem. Soc., 121:5633; and Zhou et al., 1990, Macromolecules, 23:3399). Examples of

these polyesters include poly(L-lactide-co-L-lysine) (Barrera et al., 1993, J. Am. Chem. Soc., 115:11010), poly(serine ester) (Zhou et al., 1990, Macromolecules, 23:3399), poly(4-hydroxy-L-proline ester) (Putnam et al., 1999, Macromolecules, 32:3658; and Lim et al., 1999, J. Am. Chem. Soc., 121:5633), and poly(4-hydroxy-L-proline ester) (Putnam et al., 1999, Macromolecules, 32:3658; and Lim et al., 1999, J. Am. Chem. Soc., 121:5633).

[0119] The properties of these and other polymers and methods for preparing them are well known in the art (see, for example, U.S. Pat. Nos. 6,123,727; 5,804,178; 5,770,417; 5,736,372; 5,716,404; 6,095,148; 5,837,752; 5,902,599; 5,696,175; 5,514,378; 5,512,600; 5,399,665; 5,019,379; 5,010,167; 4,806,621; 4,638,045; and 4,946,929; Wang et al., 2001, J. Am. Chem. Soc., 123:9480; Lim et al., 2001, J. Am. Chem. Soc., 123:2460; Langer, 2000, Acc. Chem. Res., 33:94; Langer, 1999, J. Control. Release, 62:7; and Uhrich et al., 1999, Chem. Rev., 99:3181). More generally, a variety of methods for synthesizing certain suitable polymers are described in Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, Ed. by Goethals, Pergamon Press, 1980; Principles of Polymerization by Odian, John Wiley & Sons, Fourth Edition, 2004; Contemporary Polymer Chemistry by Allcock et al., Prentice-Hall, 1981; Deming et al., 1997, Nature, 390:386; and in U.S. Pat. Nos. 6,506,577, 6,632,922, 6,686,446, and 6,818,732.

[0120] In some embodiments, polymers can be linear or branched polymers. In some embodiments, polymers can be dendrimers. In some embodiments, polymers can be substantially cross-linked to one another. In some embodiments, polymers can be substantially free of cross-links. In some embodiments, polymers can be used in accordance with the present invention without undergoing a cross-linking step. It is further to be understood that inventive synthetic nanocarriers may comprise block copolymers, graft copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Those skilled in the art will recognize that the polymers listed herein represent an exemplary, not comprehensive, list of polymers that can be of use in accordance with the present invention.

[0121] In some embodiments, synthetic nanocarriers may comprise metal particles, quantum dots, ceramic particles, etc. In some embodiments, a non-polymeric synthetic nanocarrier is an aggregate of non-polymeric components, such as an aggregate of metal atoms (e.g., gold atoms).

[0122] In some embodiments, synthetic nanocarriers may optionally comprise one or more amphiphilic entities. In some embodiments, an amphiphilic entity can promote the production of synthetic nanocarriers with increased stability, improved uniformity, or increased viscosity. In some embodiments, amphiphilic entities can be associated with the interior surface of a lipid membrane (e.g., lipid bilayer, lipid monolayer, etc.). Many amphiphilic entities known in the art are suitable for use in making synthetic nanocarriers in accordance with the present invention. Such amphiphilic entities include, but are not limited to, phosphoglycerides; phosphatidylcholines; dipalmitoyl phosphatidylcholine (DPPC); dioleylphosphatidyl ethanolamine (DOPE); dioleyloxypropyltriethylammonium (DOTMA); dioleoylphosphatidylcholine; cholesterol; cholesterol ester; diacylglycerol; diacylglycerolsuccinate; diphosphatidyl glycerol (DPPG); hexanedecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; fatty acids; fatty acid monoglycerides; fatty acid diglycerides; fatty acid amides; sorbitan trioleate (Span®85) glycocholate; sorbitan monolaurate (Span®20); polysorbate 20 (Tween®20); polysorbate 60 (Tween®60); polysorbate 65 (Tween®65); polysorbate 80 (Tween®80); polysorbate 85 (Tween®85); polyoxyethylene monostearate; surfactin; a poloxomer; a sorbitan fatty acid ester such as sorbitan trioleate; lecithin; lysolecithin; phosphatidylserine; phosphatidylinositol; sphingomyelin; phosphatidylethanolamine (cephalin); cardiolipin; phosphatidic acid; cerebrosides; dicetylphosphate; dipalmitoylphosphatidylglycerol; stearylamine; dodecylamine; hexadecyl-amine; acetyl palmitate; glycerol ricinoleate; hexadecyl sterate; isopropyl myristate; tyloxapol; poly(ethylene glycol)5000-phosphatidylethanolamine; poly(ethylene glycol)400-monostearate; phospholipids; synthetic and/or natural detergents having high surfactant properties; deoxycholates; cyclodextrins; chaotropic salts; ion pairing agents; and combinations thereof. An amphiphilic entity component may be a mixture of different amphiphilic entities. Those skilled in the art will recognize that this is an exemplary, not comprehensive, list of substances with surfactant activity. Any amphiphilic entity may be used in the production of synthetic nanocarriers to be used in accordance with the present invention.

[0123] In some embodiments, synthetic nanocarriers may optionally comprise one or more carbohydrates. Carbohydrates may be natural or synthetic. A carbohydrate may be a derivatized natural carbohydrate. In certain embodiments, a carbohydrate comprises monosaccharide or disaccharide, including but not limited to glucose, fructose, galactose, ribose, lactose, sucrose, maltose, trehalose, cellbiose, mannose, xylose, arabinose, glucoronic acid, galactoronic acid, mannuronic acid, glucosamine, galatosamine, and neuramic acid. In certain embodiments, a carbohydrate is a polysaccharide, including but not limited to pullulan, cellulose, microcrystalline cellulose, hydroxypropyl methylcellulose (HPMC), hydroxycellulose (HC), methylcellulose (MC), dextran, cyclodextran, glycogen, hydroxyethylstarch, carageenan, glycon, amylose, chitosan, N,O-carboxylmethylchitosan, algin and alginic acid, starch, chitin, inulin, konjac, glucommannan, pustulan, heparin, hyaluronic acid, curdlan, and xanthan. In embodiments, the inventive synthetic nanocarriers do not comprise (or specifically exclude) carbohydrates, such as a polysaccharide. In certain embodiments, the carbohydrate may comprise a carbohydrate derivative such as a sugar alcohol, including but not limited to mannitol, sorbitol, xylitol, erythritol, maltitol, and lactitol.

[0124] Compositions according to the invention comprise inventive synthetic nanocarriers in combination with pharmaceutically acceptable excipients, such as preservatives, buffers, saline, or phosphate buffered saline. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. In an embodiment, inventive synthetic nanocarriers are suspended in sterile saline solution for injection together with a preservative.

[0125] In embodiments, when preparing synthetic nanocarriers as carriers for antigens and/or adjuvants for use in vaccines, methods for coupling the antigens and/or adjuvants to the synthetic nanocarriers may be useful. If the antigen and/or adjuvant is a small molecule it may be of advantage to attach the antigen and/or adjuvant to a polymer prior to the assembly of the synthetic nanocarriers. In embodiments, it may also be an advantage to prepare the synthetic nanocarriers with sur-

face groups that are used to couple the antigen and/or adjuvant to the synthetic nanocarrier through the use of these surface groups rather than attaching the antigen and/or adjuvant to a polymer and then using this polymer conjugate in the construction of synthetic nanocarriers.

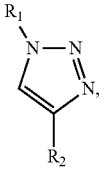
[0126] In certain embodiments, the coupling can be a covalent linker. In embodiments, peptides according to the invention can be covalently coupled to the external surface via a 1,2,3-triazole linker formed by the 1,3-dipolar cycloaddition reaction of azido groups on the surface of the nanocarrier with antigen or adjuvant containing an alkyne group or by the 1,3-dipolar cycloaddition reaction of alkynes on the surface of the nanocarrier with antigens or adjuvants containing an azido group. Such cycloaddition reactions are preferably performed in the presence of a Cu(I) catalyst along with a suitable Cu(I)-ligand and a reducing agent to reduce Cu(II) compound to catalytic active Cu(I) compound. This Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) can also be referred as the click reaction.

[0127] Additionally, the covalent coupling may comprise a covalent linker that comprises an amide linker, a disulfide linker, a thioether linker, a hydrazone linker, a hydrazide linker, an imine or oxime linker, an urea or thiourea linker, an amidine linker, an amine linker, and a sulfonamide linker.

[0128] An amide linker is formed via an amide bond between an amine on one component such as the antigen or adjuvant with the carboxylic acid group of a second component such as the nanocarrier. The amide bond in the linker can be made using any of the conventional amide bond forming reactions with suitably protected amino acids or antigens or adjuvants and activated carboxylic acid such N-hydroxysuccinimide-activated ester.

[0129] A disulfide linker is made via the formation of a disulfide (S—S) bond between two sulfur atoms of the form, for instance, of R1-S—S—R2. A disulfide bond can be formed by thiol exchange of an antigen or adjuvant containing thiol/mercaptan group (—SH) with another activated thiol group on a polymer or nanocarrier or a nanocarrier containing thiol/mercaptan groups with an antigen or adjuvant containing activated thiol group.

[0130] A triazole linker, specifically a 1,2,3-triazole of the form



wherein R1 and R2 may be any chemical entities, is made by the 1,3-dipolar cycloaddition reaction of an azide attached to a first component such as the nanocarrier with a terminal alkyne attached to a second component such as the peptide. The 1,3-dipolar cycloaddition reaction is performed with or without a catalyst, preferably with Cu(I)-catalyst, which links the two components through a 1,2,3-triazole function. This chemistry is described in detail by Sharpless et al., Angew. Chem. Int. Ed. 41(14), 2596, (2002) and Meldal, et al, Chem. Rev., 2008, 108(8), 2952-3015 and is often referred to as a "click" reaction or CuAAC.

[0131] In embodiments, a polymer containing an azide or alkyne group, terminal to the polymer chain is prepared. This polymer is then used to prepare a synthetic nanocarrier in such a manner that a plurality of the alkyne or azide groups are positioned on the surface of that nanocarrier. Alternatively, the synthetic nanocarrier can be prepared by another route, and subsequently functionalized with alkyne or azide groups. The antigen or adjuvant is prepared with the presence of either an alkyne (if the polymer contains an azide) or an azide (if the polymer contains an alkyne) group. The antigen or adjuvant is then allowed to react with the nanocarrier via the 1,3-dipolar cycloaddition reaction with or without a catalyst which covalently couples the antigen to the particle through the 1,4-disubstituted 1,2,3-triazole linker.

[0132] A thioether linker is made by the formation of a sulfur-carbon (thioether) bond in the form, for instance, of R1-S—R2. Thioether can be made by either alkylation of a thiol/mercaptan (—SH) group on one component such as the antigen or adjuvant with an alkylating group such as halide or epoxide on a second component such as the nanocarrier. Thioether linkers can also be formed by Michael addition of a thiol/mercaptan group on one component such as an antigen or adjuvant to an electron-deficient alkene group on a second component such as a polymer containing a maleimide group or vinyl sulfone group as the Michael acceptor. In another way, thioether linkers can be prepared by the radical thiol-ene reaction of a thiol/mercaptan group on one component such as an antigen or adjuvant with an alkene group on a second component such as a polymer or nanocarrier.

[0133] A hydrazone linker is made by the reaction of a hydrazide group on one component such as the antigen or adjuvant with an aldehyde/ketone group on the second component such as the nanocarrier.

[0134] A hydrazide linker is formed by the reaction of a hydrazine group on one component such as the antigen or adjuvant with a carboxylic acid group on the second component such as the nanocarrier. Such reaction is generally performed using chemistry similar to the formation of amide bond where the carboxylic acid is activated with an activating reagent.

[0135] An imine or oxime linker is formed by the reaction of an amine or N-alkoxyamine (or aminooxy) group on one component such as the antigen or adjuvant with an aldehyde or ketone group on the second component such as the nanocarrier.

[0136] An urea or thiourea linker is prepared by the reaction of an amine group on one component such as the antigen or adjuvant with an isocyanate or thioisocyanate group on the second component such as the nanocarrier.

[0137] An amidine linker is prepared by the reaction of an amine group on one component such as the antigen or adjuvant with an imidoester group on the second component such as the nanocarrier.

[0138] An amine linker is made by the alkylation reaction of an amine group on one component such as the antigen or adjuvant with an alkylating group such as halide, epoxide, or sulfonate ester group on the second component such as the nanocarrier. Alternatively, an amine linker can also be made by reductive amination of an amine group on one component such as the antigen or adjuvant with an aldehyde or ketone group on the second component such as the nanocarrier with a suitable reducing reagent such as sodium cyanoborohydride or sodium triacetoxyborohydride.

[0139] A sulfonamide linker is made by the reaction of an amine group on one component such as the antigen or adjuvant with a sulfonyl halide (such as sulfonyl chloride) group on the second component such as the nanocarrier.

[0140] A sulfone linker is made by Michael addition of a nucleophile to a vinyl sulfone. Either the vinyl sulfone or the nucleophile may be on the surface of the nanocarrier or attached to the antigen or adjuvant.

[0141] The antigen or adjuvant can also be conjugated to the nanocarrier via non-covalent conjugation methods. For example, a negative charged antigen or adjuvant can be conjugated to a positive charged nanocarrier through electrostatic adsorption. An antigen or adjuvant containing a metal ligand can also be conjugated to a nanocarrier containing a metal complex via a metal-ligand complex.

[0142] In embodiments, the antigen or adjuvant can be attached to a polymer, for example polylactic acid-block-polyethylene glycol, prior to the assembly of the synthetic nanocarrier or the synthetic nanocarrier can be formed with reactive or activatible groups on its surface. In the latter case, the antigen or adjuvant may be prepared with a group which is compatible with the attachment chemistry that is presented by the synthetic nanocarriers' surface. In other embodiments, a peptide antigen can be attached to VLPs or liposomes using a suitable linker. A linker is a compound or reagent capable of coupling two molecules together. In an embodiment, the linker can be a homobifuntional or heterobifunctional reagent as described in Hermanson 2008. For example, a VLP or liposome synthetic nanocarrier containing a carboxylic group on the surface can be treated with a homobifunctional linker, adipic dihydrazide (ADH), in the presence of EDC to form the corresponding synthetic nanocarrier with the ADH linker. The resulting ADH linked synthetic nanocarrier is then conjugated with a peptide containing an acid group via the other end of the ADH linker on NC to produce the corresponding VLP or liposome peptide conjugate.

[0143] For detailed descriptions of available conjugation methods, see Hermanson G T "Bioconjugate Techniques", 2nd Edition Published by Academic Press, Inc., 2008. In addition to covalent attachment the adjuvant can be coupled by adsorbtion to a pre-formed synthetic nanocarrier or it can be coupled by encapsulation during the formation of the synthetic nanocarrier.

[0144] In some embodiments, a component, such as an antigen or adjuvant, may be isolated. Isolated refers to the element being separated from its native environment and present in sufficient quantities to permit its identification or use. This means, for example, the element may be (i) selectively produced by expression cloning or (ii) purified as by chromatography or electrophoresis. Isolated elements may be, but need not be, substantially pure. Because an isolated element may be admixed with a pharmaceutically acceptable excipient in a pharmaceutical preparation, the element may comprise only a small percentage by weight of the preparation. The element is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e., isolated from other lipids or proteins. Any of the elements provided herein may be isolated. Any of the antigens provided herein can be included in the compositions in isolated form.

D. METHODS OF MAKING AND USING THE INVENTIVE COMPOSITIONS AND RELATED METHODS

[0145] Synthetic nanocarriers may be prepared using a wide variety of methods known in the art. For example, synthetic nanocarriers can be formed by methods as nanoprecipitation, flow focusing fluidic channels, spray drying, single and double emulsion solvent evaporation, solvent extraction, phase separation, milling, microemulsion procedures, microfabrication, nanofabrication, sacrificial layers, simple and complex coacervation, and other methods well known to those of ordinary skill in the art. Alternatively or additionally, aqueous and organic solvent syntheses for monodisperse semiconductor, conductive, magnetic, organic, and other nanomaterials have been described (Pellegrino et al., 2005, Small, 1:48; Murray et al., 2000, Ann Rev. Mat. Sci., 30:545; and Trindade et al., 2001, Chem. Mat., 13:3843). Additional methods have been described in the literature (see, e.g., Doubrow, Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992; Mathiowitz et al., 1987, J. Control. Release, 5:13; Mathiowitz et al., 1987, Reactive Polymers, 6:275; and Mathiowitz et al., 1988, J. Appl. Polymer Sci., 35:755; U.S. Pat. Nos. 5,578,325 and 6,007,845; P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010)).

[0146] Various materials may be encapsulated into synthetic nanocarriers as desirable using a variety of methods including but not limited to C. Astete et al., "Synthesis and characterization of PLGA nanoparticles" J. Biomater. Sci. Polymer Edn, Vol. 17, No. 3, pp. 247-289 (2006); K. Avgoustakis "Pegylated Poly(Lactide) and Poly(Lactide-Co-Glycolide) Nanoparticles: Preparation, Properties and Possible Applications in Drug Delivery" Current Drug Delivery 1:321-333 (2004); C. Reis et al., "Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles" Nanomedicine 2:8-21 (2006); P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010). Other methods suitable for encapsulating materials into synthetic nanocarriers may be used, including without limitation methods disclosed in U.S. Pat. No. 6,632,671 to Unger Oct. 14, 2003.

[0147] In certain embodiments, synthetic nanocarriers are prepared by a nanoprecipitation process or spray drying. Conditions used in preparing synthetic nanocarriers may be altered to yield particles of a desired size or property (e.g., hydrophobicity, hydrophilicity, external morphology, "stickiness," shape, etc.). The method of preparing the synthetic nanocarriers and the conditions (e.g., solvent, temperature, concentration, air flow rate, etc.) used may depend on the materials to be coupled to the synthetic nanocarriers and/or the composition of the polymer matrix.

[0148] If particles prepared by any of the above methods have a size range outside of the desired range, particles can be sized, for example, using a sieve.

[0149] Elements of the inventive synthetic nanocarriers may be coupled to the overall synthetic nanocarrier, e.g., by one or more covalent bonds, or may be coupled by means of one or more linkers. Additional methods of functionalizing synthetic nanocarriers may be adapted from Published US Patent Application 2006/0002852 to Saltzman et al., Pub-

lished US Patent Application 2009/0028910 to DeSimone et al., or Published International Patent Application WO/2008/127532 A1 to Murthy et al.

[0150] Alternatively or additionally, synthetic nanocarriers can be coupled to elements directly or indirectly via non-covalent interactions. In non-covalent embodiments, the non-covalent coupling is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, TT stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. Such couplings may be arranged to be on an external surface or an internal surface of an inventive synthetic nanocarrier. In embodiments, encapsulation and/or absorption is a form of coupling.

[0151] In embodiments, the inventive synthetic nanocarriers can be combined with other adjuvants by admixing in the same vehicle or delivery system. Such adjuvants may include, but are not limited to mineral salts, such as alum, alum combined with monphosphoryl lipid (MPL) A of Enterobacteria, such as *Escherihia coli, Salmonella minnesota, Salmonella typhimurium*, or *Shigella flexneri* or specifically with MPL® (AS04), MPL A of above-mentioned bacteria separately, saponins, such as QS-21, Quil-A, ISCOMs, ISCOMATRIX™, emulsions such as MF59™, Montanide® ISA 51 and ISA 720, AS02 (QS21+ squalene+MPL®), liposomes and liposomal formulations such as AS01, synthesized or specifically prepared microparticles and microcarriers such as bacteria-derived outer membrane vesicles (OMV) of *N. gonorrheae, Chlamydia trachomatis* and others, or chitosan particles, depot-forming agents, such as Pluronic® block co-polymers, specifically modified or prepared peptides, such as muramyl dipeptide, aminoalkyl glucosaminide 4-phosphates, such as RC529, or proteins, such as bacterial toxoids or toxin fragments. The doses of such other adjuvants can be determined using conventional dose ranging studies.

[0152] In embodiments, the inventive synthetic nanocarriers can be combined with an antigen different, similar or identical to those coupled to a nanocarrier (with or without adjuvant, utilizing or not utilizing another delivery vehicle) administered separately at a different time-point and/or at a different body location and/or by a different immunization route or with another antigen and/or adjuvant-carrying synthetic nanocarrier administered separately at a different time-point and/or at a different body location and/or by a different immunization route.

[0153] Populations of synthetic nanocarriers may be combined to form pharmaceutical dosage forms according to the present invention using traditional pharmaceutical mixing methods. These include liquid-liquid mixing in which two or more suspensions, each containing one or more subset of nanocarriers, are directly combined or are brought together via one or more vessels containing diluent. As synthetic nanocarriers may also be produced or stored in a powder form, dry powder-powder mixing could be performed as could the resuspension of two or more powders in a common media. Depending on the properties of the nanocarriers and their interaction potentials, there may be advantages conferred to one or another route of mixing.

[0154] Typical compositions that comprise synthetic nanocarriers may comprise inorganic or organic buffers (e.g., sodium or potassium salts of phosphate, carbonate, acetate, or citrate) and pH adjustment agents (e.g., hydrochloric acid, sodium or potassium hydroxide, salts of citrate or acetate, amino acids and their salts) antioxidants (e.g., ascorbic acid, alpha-tocopherol), surfactants (e.g., polysorbate 20, polysorbate 80, polyoxyethylene9-10 nonyl phenol, sodium desoxycholate), solution and/or cryo/lyo stabilizers (e.g., sucrose, lactose, mannitol, trehalose), osmotic adjustment agents (e.g., salts or sugars), antibacterial agents (e.g., benzoic acid, phenol, gentamicin), antifoaming agents (e.g., polydimethylsilozone), preservatives (e.g., thimerosal, 2-phenoxyethanol, EDTA), polymeric stabilizers and viscosity-adjustment agents (e.g., polyvinylpyrrolidone, poloxamer 488, carboxymethylcellulose) and co-solvents (e.g., glycerol, polyethylene glycol, ethanol).

[0155] Compositions according to the invention comprise synthetic nanocarriers in combination with pharmaceutically acceptable excipients. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. Techniques suitable for use in practicing the present invention may be found in Handbook of Industrial Mixing: Science and Practice, Edited by Edward L. Paul, Victor A. Atiemo-Obeng, and Suzanne M. Kresta, 2004 John Wiley & Sons, Inc.; and Pharmaceutics: The Science of Dosage Form Design, 2nd Ed. Edited by M. E. Auten, 2001, Churchill Livingstone. In an embodiment, inventive synthetic nanocarriers are suspended in sterile saline solution for injection together with a preservative.

[0156] It is to be understood that the compositions of the invention can be made in any suitable manner, and the invention is in no way limited to compositions that can be produced using the methods described herein. Selection of an appropriate method may require attention to the properties of the particular moieties being associated.

[0157] In some embodiments, the synthetic nanocarriers are manufactured under sterile conditions or are terminally sterilized. This can ensure that resulting composition are sterile and non-infectious, thus improving safety when compared to non-sterile compositions. This provides a valuable safety measure, especially when subjects receiving synthetic nanocarriers have immune defects, are suffering from infection, and/or are susceptible to infection. In some embodiments, inventive synthetic nanocarriers may be lyophilized and stored in suspension or as lyophilized powder depending on the formulation strategy for extended periods without losing activity.

[0158] The compositions of the invention can be administered by a variety of routes, including or not limited to subcutaneous, intranasal, oral, intravenous, intraperitoneal, intramuscular, transmucosal, transmucosal, sublingual, rectal, ophthalmic, pulmonary, intradermal, transdermal, transcutaneous or intradermal or by a combination of these routes. Routes of administration also include administration by inhalation or pulmonary aerosol. Techniques for preparing aerosol delivery systems are well known to those of skill in the art (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp. 1694-1712; incorporated by reference).

[0159] Doses of dosage forms contain varying amounts of populations of synthetic nanocarriers and/or varying amounts of antigens, adjuvants, etc., according to the invention. The amount of synthetic nanocarriers and/or other elements present in the inventive dosage forms can be varied according to the nature of the elements, the therapeutic benefit to be

accomplished, and other such parameters. In embodiments, dose ranging studies can be conducted to establish optimal therapeutic amount of the population of synthetic nanocarriers and the amount of antigens to be present in the dosage form. In embodiments, the synthetic nanocarriers and the antigens are present in the dosage form in an amount effective to generate an immune response to the antigens upon administration to a subject. It may be possible to determine amounts of the antigens effective to generate an immune response using conventional dose ranging studies and techniques in subjects. Inventive dosage forms may be administered at a variety of frequencies. In a preferred embodiment, at least one administration of the dosage form is sufficient to generate a pharmacologically relevant response. In more preferred embodiment, at least two administrations, at least three administrations, or at least four administrations, of the dosage form are utilized to ensure a pharmacologically relevant response.

[0160] The compositions and methods described herein can be used to induce, enhance, suppress, modulate, direct, or redirect an immune response. The compositions and methods described herein can be used in the diagnosis, prophylaxis and/or treatment of conditions such as cancers, infectious diseases, metabolic diseases, degenerative diseases, non-autoimmune diseases or other disorders and/or conditions. The compositions and methods described herein can also be used for the prophylaxis or treatment of an addiction, such as an addiction to an illegal drug, an over-the-counter drug, a prescription drug. In some embodiments, the addiction is to cocaine, heroin, marijuana, methamphetamines, nicotine or a narcotic. The compositions and methods described herein can also be used for the prophylaxis and/or treatment of a condition resulting from the exposure to a toxin, hazardous substance, environmental toxin, or other harmful agent.

[0161] Examples of infectious disease include, but are not limited to, viral infectious diseases, such as AIDS, Chickenpox (Varicella), Common cold, Cytomegalovirus Infection, Colorado tick fever, Dengue fever, Ebola hemorrhagic fever, Hand, foot and mouth disease, Hepatitis, Herpes simplex, Herpes zoster, HPV, Influenza (Flu), Lassa fever, Measles, Marburg hemorrhagic fever, Infectious mononucleosis, Mumps, Norovirus, Poliomyelitis, Progressive multifocal leukencephalopathy, Rabies, Rubella, SARS, Smallpox (Variola), Viral encephalitis, Viral gastroenteritis, Viral meningitis, Viral pneumonia, West Nile disease and Yellow fever; bacterial infectious diseases, such as Anthrax, Bacterial Meningitis, Botulism, Brucellosis, Campylobacteriosis, Cat Scratch Disease, Cholera, Diphtheria, Epidemic Typhus, Gonorrhea, Impetigo, Legionellosis, Leprosy (Hansen's Disease), Leptospirosis, Listeriosis, Lyme disease, Melioidosis, Rheumatic Fever, MRSA infection, Nocardiosis, Pertussis (Whooping Cough), Plague, Pneumococcal pneumonia, Psittacosis, Q fever, Rocky Mountain Spotted Fever (RMSF), Salmonellosis, Scarlet Fever, Shigellosis, Syphilis, Tetanus, Trachoma, Tuberculosis, Tularemia, Typhoid Fever, Typhus and Urinary Tract Infections; parasitic infectious diseases, such as African trypanosomiasis, Amebiasis, Ascariasis, Babesiosis, Chagas Disease, Clonorchiasis, Cryptosporidiosis, Cysticercosis, Diphyllobothriasis, Dracunculiasis, Echinococcosis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Free-living amebic infection, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Kala-azar, Leishmaniasis, Malaria, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Pinworm Infection, Scabies, Schistosomiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinellosis, Trichinosis, Trichuriasis, Trichomoniasis and Trypanosomiasis; fungal infectious disease, such as Aspergillosis, Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Tinea pedis (Athlete's Foot) and Tinea cruris; prion infectious diseases, such as Alpers' disease, Fatal Familial Insomnia, Gerstmann-Sträussler-Scheinker syndrome, Kuru and Variant Creutzfeldt-Jakob disease.

[0162] Examples of cancers include, but are not limited to breast cancer; biliary tract cancer; bladder cancer; brain cancer including glioblastomas and medulloblastomas; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia, e.g., B Cell CLL; T-cell acute lymphoblastic leukemia/lymphoma; hairy cell leukemia; chronic myelogenous leukemia, multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia/lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Merkel cell carcinoma, Kaposi's sarcoma, basal cell carcinoma, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma (teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor.

[0163] Examples of metabolic diseases include, but are not limited to, disorders of carbohydrate metabolism, amino acid metabolism, organic acid metabolism, fatty acid oxidation and mitochondrial metabolism, prophyrin metabolism, purine or pyrimidine metabolism, steroid metabolism, lysosomal mitochondrial function, peroxisomal function, lysosomal storage, urea cycle disorders (e.g., N-acetyl glutamate synthetase deficiency, carbamylphosphate synthase deficiency, ornithine carbamyl transferase deficiency, crginosuccinic aciduria, citrullinaemia, arginase deficiency), amino acid disorders (e.g., Non-ketotic hyperglycinaemia, tyrosinaemia (Type I), Maple syrup urine disease), organic acidemias (e.g, isovaleric acidemia, methylmalonic acidemia, propionic acidemia, glutaric aciduria type I, glutaric acidemia type I & II), mitochondrial disorders (e.g., carboxylase defects, mitochondrial myopathies, lactic acidosis (pyruvate dehydrogenase complex defects), congenital lactic acidosis, mitochondrial respiratory chain defects, cystinosis, Gaucher's disease, Fabry's disease, Pompe's disease, mucopolysaccharoidosis I, mucopolysaccharoidosis II, mucopolysaccharoidosis VI).

[0164] Examples of degenerative diseases include, but are not limited to, mesenchyme/mesoderm degenerative disease, muscle degenerative disease, endothelial degenerative disease, neurodegenerative disease, degenerative joint disease (e.g., osteoarthritis), major types of degenerative heart disease (e.g., coronary heart disease, congenital heart disease, rheumatic heart disease, angina pectoris), neurodegenerative disease (e.g., Alzheimer's disease, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, spinal muscular atrophy), neu-

romuscular disorders (e.g., muscular dystrophy, duchenne muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic muscular dystrophy, congenital myopathy, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease).

EXAMPLES

Example 1

Formulations of Synthetic Nanocarriers

Materials for Lot #1

[0165] Ovalbumin peptide 323-339 amide acetate salt, was purchased from Bachem Americas Inc. (3132 Kashiwa Street, Torrance Calif. 90505. Part #4065609.) PLGA-R848 conjugate of 75/25 lactide/glycolide monomer composition and approximately 4100 Da molecular weight having 5.2% w/w R848 content was synthesized by conjugation of R848 to the terminal-acid of commercially-supplied PLGA via an amide linkage. PLA-PEG-Nicotine with a nicotine-terminated PEG block of 3,500 Da and DL-PLA block of approximately 15,000 Da was synthesized. Polyvinyl alcohol (Mw=9,000-10,000, 80% hydrolyzed) was purchased from SIGMA (Part Number 360627).

Methods for Lot #1

[0166] Solutions were prepared as follows:

[0167] Solution 1: Ovalbumin peptide 323-339 amide acetate salt @ 70 mg/mL was prepared by dissolution in 0.13N hydrochloric acid at room temperature.

[0168] Solution 2: PLGA-R848 @ 75 mg/mL and PLA-PEG-Nicotine @ 25 mg/mL in dichloromethane was prepared by dissolving PLGA-R848 at 100 mg/mL in dichloromethane and PLA-PEG-Nicotine at 100 mg/mL in dichloromethane, then combining 3 parts of the PLGA-R848 solution to 1 part of the PLA-PEG-Nicotine solution.

[0169] Solution 3: Polyvinyl alcohol @ 50 mg/mL in 100 mM phosphate buffer, pH 8.

[0170] Solution 4: 70 mM phosphate buffer, pH 8.

[0171] A primary (W1/O) emulsion was first created using Solution 1 & Solution 2. Solution 1 (0.1 mL) and Solution 2 (1.0 mL) were combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion was then formed by adding Solution 3 (2 mL) to the primary emulsion and sonicating at 30% amplitude for 40 seconds using the Branson Digital Sonifier 250. The secondary emulsion was added to an open 50 mL beaker containing 30 mL of stiffing 70 mM phosphate buffer solution and was stirred at room temperature for not less than 2 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 13800 rcf for 60 minutes at 4° C., removing the supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure was repeated and then the pellet was re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension which was stored frozen at −20C until use.

TABLE 1

Nanocarrier ID	Effective Diameter (nm)	TLR Agonist, % w/w	T-cell helper peptide, % w/w
1	197	1.5	0.8

Materials for Lot #2

[0172] Ovalbumin peptide 323-339 amide acetate salt, was purchased from Bachem Americas Inc. (3132 Kashiwa Street, Torrance Calif. 90505. Part #4065609.) PLGA-R848 conjugate of 75/25 lactide/glycolide monomer composition and approximately 4100 Da molecular weight having 5.2% w/w R848 content was synthesized by conjugation of R848 to the terminal-acid of commercially-supplied PLGA via an amide linkage. PLA with an inherent viscosity of 0.19 dL/g was purchased from Boehringer Ingelheim (Ingelheim Germany. Product Code R202H). PLA-PEG-Nicotine with a nicotine-terminated PEG block of 3,500 Da and DL-PLA block of approximately 15,000 Da was synthesized. Polyvinyl alcohol (Mw=9,000-10,000, 80% hydrolyzed) was purchased from SIGMA (Part Number 360627).

Methods for Lot #2

[0173] Solutions were prepared as follows:

[0174] Solution 1: Ovalbumin peptide 323-339 amide acetate salt @ 70 mg/mL was prepared by dissolution in 0.13N hydrochloric acid at room temperature.

[0175] Solution 2: PLGA-R848 @ 75 mg/mL, PLA-PEG-Nicotine @ 6 mg/mL, and PLA at 19 mg/mL in dichloromethane was prepared by dissolving PLGA-R848 at 100 mg/mL in dichloromethane, PLA-PEG-Nicotine at 100 mg/mL in dichloromethane, and PLA at 100 mg/mL in dichloromethane and then combining 750 μL of the PLGA-R848 solution with 60 μL of the PLA-PEG-Nicotine solution and 190 μL of the PLA solution.

[0176] Solution 3: Polyvinyl alcohol @ 50 mg/mL in 100 mM phosphate buffer, pH 8.

[0177] Solution 4: 70 mM phosphate buffer, pH 8.

[0178] A primary (W1/O) emulsion was first created using Solution 1 & Solution 2. Solution 1 (0.1 mL) and Solution 2 (1.0 mL) were combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion was then formed by adding Solution 3 (2 mL) to the primary emulsion and sonicating at 10% amplitude for 40 seconds using the Branson Digital Sonifier 250. The secondary emulsion was added to an open 50 mL beaker containing 30 mL of stiffing 70 mM phosphate buffer solution and was stirred at room temperature for not less than 2 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 13800 rcf for 60 minutes at 4° C., removing the supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure was repeated and then the pellet was re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension which was stored frozen at −20C until use.

TABLE 2

Nanocarrier ID	Effective Diameter (nm)	TLR Agonist, % w/w	T-cell helper peptide, % w/w
2	212	1.4	1.8

TABLE 3

Nanocarrier ID	Effective Diameter (nm)	TLR Agonist, % w/w	T-cell helper peptide, % w/w
3	197	1.8	0.9

Materials for Lot #3

[0179] Ovalbumin peptide 323-339 amide acetate salt, was purchased from Bachem Americas Inc. (3132 Kashiwa Street, Torrance Calif. 90505. Part #4065609.) PLGA-R848 conjugate of 75/25 lactide/glycolide monomer composition and approximately 4100 Da molecular weight having 5.2% w/w R848 content was synthesized by conjugation of R848 to the terminal-acid of commercially-supplied PLGA via an amide linkage. PLA-PEG-Nicotine with a nicotine-terminated PEG block of 3,500 Da and DL-PLA block of approximately 15,000 Da was synthesized. PLA-PEG-OMe block co-polymer with a PEG-OMe (Methyl-ether capped PEG) block of 2,000 Da and DL-PLA block of approximately 19,000 Da was synthesized. Polyvinyl alcohol (Mw=9,000-10,000, 80% hydrolyzed) was purchased from SIGMA (Part Number 360627).

Methods for Lot #3

[0180] Solutions were prepared as follows:

[0181] Solution 1: Ovalbumin peptide 323-339 amide acetate salt @ 70 mg/mL was prepared by dissolution in 0.13N hydrochloric acid at room temperature.

[0182] Solution 2: PLGA-R848 @ 75 mg/mL, PLA-PEG-Nicotine @ 6 mg/mL, and PLA-PEG-OMe at 19 mg/mL in dichloromethane was prepared by dissolving PLGA-R848 at 100 mg/mL in dichloromethane, PLA-PEG-Nicotine at 100 mg/mL in dichloromethane, and PLA-PEG-OMe at 100 mg/mL in dichloromethane and then combining 750 μL of the PLGA-R848 solution with 60 μL of the PLA-PEG-Nicotine solution and 190 μL of the PLA-PEG-OMe solution.

[0183] Solution 3: Polyvinyl alcohol @ 50 mg/mL in 100 mM phosphate buffer, pH 8.

[0184] Solution 4: 70 mM phosphate buffer, pH 8.

[0185] A primary (W1/O) emulsion was first created using Solution 1 & Solution 2. Solution 1 (0.1 mL) and Solution 2 (1.0 mL) were combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion was then formed by adding Solution 3 (2 mL) to the primary emulsion and sonicating at 10% amplitude for 40 seconds using the Branson Digital Sonifier 250. The secondary emulsion was added to an open 50 mL beaker containing 30 mL of stiffing 70 mM phosphate buffer solution and was stirred at room temperature for not less than 2 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 13800 rcf for 60 minutes at 4° C., removing the supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure was repeated and then the pellet was re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension which was stored frozen at −20C until use.

Materials for Lots #4-#12

[0186] Ovalbumin peptide 323-339 amide acetate salt, was purchased from Bachem Americas Inc. (3132 Kashiwa Street, Torrance Calif. 90505. Product code 4065609.) PLGA-R848 of approximately 5,200 Da made from PLGA of 3:1 lactide to glycolide ratio and having 12.7% w/w conjugated R848 content was synthesized. PLA with an inherent viscosity of 0.21 dL/g was purchased from SurModics Pharmaceuticals (756 Tom Martin Drive, Birmingham, Ala. 35211. Product Code 100 DL 2A.) PLA-PEG_{2k}-OMe block co-polymer with a methyl ether terminated PEG block of 2,000 Da and DL-PLA block of approximately 19,000 Da was synthesized. PLA-PEG_{5k}-OMe block co-polymer with a methyl ether terminated PEG block of 5,000 Da and DL-PLA block of approximately 20,000 Da was synthesized. PLA-PEG-Nicotine block copolymer having a nicotine-terminated PEG block of 5,000 Da and DL-PLA block of approximately 21,000 Da was synthesized. Polyvinyl alcohol (Mw=11,000-31,000, 87-89% hydrolyzed) was purchased from J. T. Baker (Part Number U232-08).

Methods for Lots #4-#12

[0187] Solutions were prepared as follows:

[0188] Solution 1: Ovalbumin peptide 323-339 amide acetate salt @ 20 mg/mL was prepared by dissolution in 0.13N hydrochloric acid at room temperature.

[0189] Solution 2: Stock solutions, each containing one of the individual polymers (PLGA-R848, PLA, PLA-PEG_{2k}-OMe, PLA-PEG_{5k}-OMe, and PLA-PEG-Nicotine), were prepared in dichloromethane at 100 mg/mL. These single-polymer stocks were combined according to Table 4 to generate a unique "Solution 2" for each of the nanocarrier lots.

[0190] Solution 3: Polyvinyl alcohol @ 50 mg/mL in 100 mM in 100 mM phosphate buffer, pH 8.

[0191] Solution 4: 70 mM phosphate buffer, pH 8.

[0192] A primary (W1/O) emulsion was first created using Solution 1 & Solution 2. Solution 1 (0.2 mL) and Solution 2 (1.0 mL) were combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion was then formed by adding Solution 3 (2.0 mL) to the primary emulsion, vortexing to create a course dispersion, and then sonicating at 30% amplitude for 40 seconds using the Branson Digital Sonifier 250. The secondary emulsion was added to an open 50 mL beaker containing 70 mM phosphate buffer solution (30 mL) and stirred at room temperature for 2 to 3 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 21,000 rcf for 45 minutes, removing the supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure was repeated and then the pellet was re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension was stored frozen at −20° C. until use.

TABLE 4

Composition of Solution 2 for Nanocarrier Formulation

		Solution 2 Composition for Production of Example Nanocarrier Lots								
		4	6	7	10	8	9	5	12	11
Polymer Solution (100 mg/mL)	PLA-PEG-Nicotine	0.25 mL	0.50 mL	0.25 mL	0.25 mL	0.375 mL	0.375 mL	0.375 mL	—	—
	PLA-PEG$_{2k}$-OMe	—	—	0.25 mL	—	0.125 mL	—	—	—	—
	PLA-PEG$_{5k}$-OMe				0.25 mL	—	0.125 mL	—	—	0.50 mL
	PLA	0.25 mL	—	—	—	—	—	0.125 mL	0.50 mL	—
	PLGA-R848	0.50 mL	0.50 mL	0.50 mL	0.50 mL	0.50 mL	0.50 mL	0.50 mL	0.50 mL	0.50 mL

TABLE 5

Gr.	NC Lot #	PLA-PEG-Nic (% w/w)	PLA-PEG$_{2k}$-OMe (% w/w)	PLA-PEG$_{5k}$-OMe (% w/w)	PLA (% w/w)	Ova Peptide Load (% w/w)	R848 Load (% w/w)
1	4	25	0	0	25	2.0	4.1
3	6	50	0	0	0	1.0	3.9
4	7	25	25	0	0	1.1	3.9
7	10	25	0	25	0	0.1	4.0
5	8	37.5	12.5	0	0	1.0	4.4
6	9	37.5	0	12.5	0	0.7	4.1
2	5	37.5	0	0	12.5	1.8	4.4
9	12	0	0	0	50	0.7	4.2
8	11	0	0	50	0	0	4.6

Example 2

Synthetic Nanocarriers with Increased Antigen Increases Antigen-Specific Antibody Generation and Decreases Anti-Carrier Antibody Generation

[0193] Mice were inoculated with nicotine-presenting R848-adjuvanted nanocarrier formulations. Groups 2 through 4 were evaluated for antigen-presentation and anti-carrier effect. The nicotine-presenting conjugate in the nanocarrier is a PLA-PEG3.5k-Nicotine construct of ~15,350 Mw PLA and ~3500 Mw PEG. The study groups used formulations having varied content of the PLA-PEG3.5k-Nicotine construct, partially-substituting the construct with either a ~20 k Mw PLA polymer or with a PLA-PEG2k-OMe polymer of ~18,700 Mw PLA and 2000 Mw PEG. Mice were immunized at days 0, 14, and 28 and serum was collected at days 26 and 40. The formulations are described as tabulated below and the anti-nicotine and resultant anti-PEG antibodies at day 40 are presented in FIG. **1**.

TABLE 6

Synthetic Nanocarrier Formulations

Gr.	NC Lot #	PLGA-R848 Conjugate, % of NC mass	Polymer-Ag Description, % of NC mass	Replacement polymer, % of NC mass	R848 Load (%)	Ova Peptide Load (%)	μg R848/mg NP released (24 hrs, citrate pH 4.5)
2	1	75%	PLA-PEG-Nic, 25%	None	1.5	0.8	3.7
3	2	75%	PLA-PEG-Nic, 6%	R202H PLA, 19%	1.4	1.8	3.9
4	3	75%	PLA-PEG-Nic, 6%	PLA-PEG, 19%	1.8	0.9	4.5

[0194] Antibody titers to nicotine and PEG were determined by ELISA using sera collected from immunized mice. Plates were coated with 100 μL per well of either polylysine-nicotine (PLL-Nic), PLA-PEG-OMe, or polylysine-PEG (PLL-PEG-OMe) and incubated overnight at 4° C. Plates were washed three times with wash buffer (0.05% Tween-20 in PBS) and blocked at room temperature for two hours using 200 μL per well of 10% fetal bovine serum (FBS) in PBS (diluent). Serum samples were added to the wells of the top row of a 96-well plate and diluted 3-fold down the plate to obtain an antibody titration curve. For a positive control, either a mouse anti-nicotine monoclonal antibody or a biotinylated rabbit anti-PEG monoclonal antibody (Epitomics, Catalog #2137-1) were used in two columns of the plate. For negative controls, either serum from unimmunized mice or isotype control antibodies were used. Plates were incubated for two hours at room temperature and washed three times with wash buffer. Secondary detection antibody (biotinylated goat anti-mouse Ig, BD Biosciences, Catalog #553999) was diluted 1:1000 in diluent and 100 μL was added to each well of the plate. Plates were incubated for one hour at room temperature and washed three times with wash buffer. Detection enzyme (streptavidin-horseradish peroxidase, SA-HRP, BD Biosciences, Catalog #554066) was diluted 1:1000 in diluent and 100 μL was added to each well of the plate. Plates were incubated for 30 minutes at room temperature in the dark and washed three times with wash buffer (during each wash step, plates were incubated with wash buffer for at least 30 seconds). TMB substrate (BD Biosciences, Catalog #555214) was added to the plate (100 μL per well) and incubated for 15 minutes at room temperature in the dark. Stop solution (2N sulfuric acid) was added to stop the enzymatic reaction (50 μL per well) and the optical density of the plates was read using a plate reader at 450 nm wavelength with subtraction of 570 nm. The half maximal effective concentration (EC50) of antibodies was calculated based on the generated four-parameter logistic curve-fit graph. The average OD value of two diluent-only blanks (negative control) was subtracted from the rest of the wells of the plate. The EC50 value of the average top OD value of the two standards was used to determine the EC50 value for the rest of the plate.

[0195] The data show a non-linear increase in anti-nicotine (target) antibodies with higher nicotine content of the nanocarrier (25% vs. 6% PLA-PEG3.5k-Nicotine); a 5-fold increase in PLA-PEG3.5k-Nicotine yielded a 21 to 74-fold higher anti-nicotine response while achieving a ~31:1 ratio of anti-nicotine to anti-PEG antibodies. Surprisingly, in the case where ~75% of the PLA-PEG3.5k-Nicotine was substituted with PLA-PEG2k-OMe, the anti-PEG titer exceeded the nicotine titer to yield a 1:10 ratio of anti-nicotine to anti-PEG antibodies. Anti-PEG antibody titers were 8-fold higher in formulations containing 6% PLA-PEG3.5k-Nicotine than those containing 25% PLA-PEG3.5k-Nicotine. Additionally, in the group that was inoculated with Lot 2 (contained 19% PLA polymer instead of 19% PLA-PEG2k-OMe), anti-PEG antibody levels were nearly absent.

Example 3

Synthetic Nanocarriers with Increased Antigen or Increased Polymer Length Decreases Anti-Carrier Antibody Generation

[0196] Mice were inoculated with nicotine-presenting R848-adjuvanted nanocarrier formulations. All formulations were prepared on the same date using a consistent set of solutions and materials. All tested nanocarriers were formulated with a 50% PLGA-R848 polymer content, with the remaining 50% of the composition made up of one or more of the following polymers: PLA-PEG5k-Nicotine, PLA-PEG2k-OMe, PLA-PEG5k-OMe, or PLA.

TABLE 7

Synthetic Nanocarrier Formulations

Gr.	NC Lot #	PLA-PEG-Nic (% w/w)	PLA-PEG$_{2k}$-OMe (% w/w)	PLA-PEG$_{5k}$-OMe (% w/w)	PLA (% w/w)	Ova Peptide Load (% w/w)	R848 Load (% w/w)
1	4	25	0	0	25	2.0	4.1
2	5	37.5	0	0	12.5	1.8	4.4
3	6	50	0	0	0	1.0	3.9
4	7	25	25	0	0	1.1	3.9
5	8	37.5	12.5	0	0	1.0	4.4
6	9	37.5	0	12.5	0	0.7	4.1
7	10	25	0	25	0	0.1	4.0
8	11	0	0	50	0	0	4.6
9	12	0	0	0	50	0.7	4.2

[0197] Following a prime and two-boost inoculation schedule, the on-target (anti-nicotine) antibody titers and off-target (anti-PEG) antibody titers were determined by ELISA as described above (except PEG length in the ELISA coating materials was adjusted to match the length used in the nanoparticles used for injections when applicable) and are presented in FIG. **2**.

[0198] The results revealed several surprising outcomes with implications on nanocarrier vaccine formulations. For example, at 25% PLA-PEG5k-Nicotine content or higher, with no other sources of PEG in the formulation, essentially no induction of anti-PEG antibodies is observed. Additionally, the incorporation of PLA-PEG5k-Nicotine above 25% of the particle content does not further increase anti-nicotine antibody titers (plateau effect). Incorporation of 100% more nicotine actually resulted in a decrease in anti-nicotine antibody titers. Introduction of shorter-chain filler PLA-PEG2k (PEG of 2000 Mw) leads to a significant anti-PEG antibody titer, whereas a longer-PEG-chain filler PLA-PEG5k (PEG of 5000 Mw) results in limited anti-PEG titers. This result is evident at two different content levels of the filler introduction whether the anti-PEG response is considered as an absolute titer or as a ratio to the intended anti-nicotine response.

1. A composition comprising:

a population of synthetic nanocarriers, wherein the synthetic nanocarriers comprise (i) a B cell antigen and (ii)

an off-target response attenuating polymeric coating, wherein the B cell antigen is coupled to the synthetic nanocarrier.

2. The composition of claim **1**, wherein the coating comprises one or more polymers present at at least a portion of the surface of the synthetic nanocarriers.

3. The composition of claim **1**, wherein the B cell antigen is coupled to the off-target response attenuating polymeric coating.

4. The composition of claim **1**, wherein the off-target response attenuating polymeric coating comprises a polymer with a weight average or number average molecular weight of greater than 2000 g/mole, of greater than 3000 g/mole, of greater than 4000 g/mole, of greater than 5000 g/mole, of between 3500 g/mole and 5000 g/mole, or of 5000 g/mole.

5.-**9**. (canceled)

10. The composition of claim **1**, wherein the B cell antigen is coupled to the polymer.

11. The composition of claim **1**, wherein the off-target response attenuating polymeric coating comprises another polymer.

12. The composition of claim **11**, wherein the B cell antigen is coupled to the other polymer.

13.-**18**. (canceled)

19. The composition of claim **11**, wherein the polymer and other polymer are the same type of polymer.

20. The composition of claim **11**, wherein the polymer and other polymer are not the same type of polymer.

21. The composition of claim **1**, wherein the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers, or the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers is between 0.001 and 1, between 0.01 and 1, between 0.1 and 1, between 0.25 and 1, between 0.5 and 1, or between 0.75 and 1.

22.-**26**. (canceled)

27. The composition of claim **21**, wherein the ratio is based on the polymeric coating across the population of synthetic nanocarriers, or on the synthetic nanocarrier as a whole across the population of synthetic nanocarriers.

28. (canceled)

29. The composition of claim **1**, wherein the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer not coupled to the B cell antigen, the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen, or the ratio by weight averaged across the population of synthetic nanocarriers of polymer not coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen is between 0.1 and 1, between 0.25 and 1, between 0.5 and 1, between 0.1 and 0.5, or 0.5.

30.-**35**. (canceled)

36. The composition of claim **1** or claim **11**, wherein the polymer and/or other polymer comprises polyethylene glycol, a polyethyloxazoline, a polyamino acid, polycarbonate, hydrophilic polyacetal, hydrophilic polyketal, saccharide polypropylene, or polyethyleneimine.

37.-**38**. (canceled)

39. The composition of claim **1**, wherein the B cell antigen comprises a protein, peptide, small molecule or oligosaccharide.

40. (canceled)

41. The composition of claim **1**, wherein the composition further comprises a T cell antigen.

42. (canceled)

43. The composition of claim **1**, wherein the composition further comprises an adjuvant and/or a pharmaceutically acceptable excipient.

44. (canceled)

45. A dosage form comprising the composition of claim **1**.

46. A vaccine comprising the dosage form of claim **45**.

47. A method comprising administering the dosage form of claim **45** to a subject in need thereof.

48.-**49**. (canceled)

50. A process for producing a synthetic nanocarrier comprising an off-target response attenuating polymeric coating, comprising the steps of: (a) providing a composition comprising one or more polymers present at at least a portion of the surface of a synthetic nanocarrier; (b) coupling a B cell antigen to said synthetic nanocarrier under conditions where: (i) the molecular weight of the polymers (as weight average or number average molecular weight); and/or (ii) the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (iii) the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer not coupled to the B cell antigen; and/or (iv) the ratio of the average number of polymers coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (v) the ratio by weight averaged across the population of synthetic nanocarriers of polymer coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen; and/or (vi) the ratio of the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers to the average number of polymers coupled to the B cell antigen plus the average number of polymers not coupled to the B cell antigen across the population of synthetic nanocarriers; and/or (vii) the ratio by weight averaged across the population of synthetic nanocarriers of polymer not coupled to the B cell antigen nanocarriers to polymer coupled to the B cell antigen plus polymer not coupled to the B cell antigen; are selected such that an antibody response against the B cell antigen is at least two-fold greater than an off-target antibody response.

51.-**59**. (canceled)

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