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(54) Title: COMPOSITION

(57) Abstract: An immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, wherein the composition is capable of eliciting an immune response to the first antigen when administered to a human or non-human animal. Uses of the immunogenic composition, and a pharmaceutical composition or a kit comprising the immunogenic composition.

COMPOSITION

The present invention relates to a composition for use in eliciting an immune response. In particular, the invention relates to an immunogenic composition that focuses a B-cell immune response to a specific region of an antigen. The invention also relates to the use of immunogenic compositions and to methods of eliciting immunogenic responses.

Immunisation of a human or non-human animal with a protein antigen generally results in a very polyclonal antibody response directed to many of the exposed protein surfaces. A polyclonal response is a natural response which ensures that an antigen is recognised by different antibodies which recognise different epitopes on the antigen. However, it is known that in some infections, such as HIV-1 infection, much of the antibody response in a polyclonal response to the virus is non-neutralising. However, a few rare individuals do have a response that produces broadly neutralising sera, and in these cases the antibody response is more focussed in nature with most antibodies being directed to a specific epitope. In the case of HIV-1 infections, epitopes which produce broadly neutralising antisera has been mapped to the CD4 binding site on the gp120 protein, as recognised by neutralising antibodies b12, VRC01, HJ16 (Zhou T. et al., Science. 2010 Aug 13;329(5993):770-3), and to the V-loops, as recognised by neutralising antibodies PG9 and PG16 (Walker L M et al., PLoS Pathog. 2010 Aug 5;6(8)).

Whilst focusing of the immune response has been observed in a few individuals, the problem remains as to how to elicit such a focussed antibody response using a vaccine composition. The problem lies in how to present the epitope or epitopes of interest in the correct confirmation to focus the immune response, to avoid a polyclonal response and to generate neutralising antibodies. Efforts have been made to focus the immune response, in particular with respect to HIV-1, where attempts have been made to constrain the structure with disulphide bridges so as to expose the CD4 binding site, or to transplant the binding site structure to other protein scaffolds, but all have proven unsuccessful.

There therefore remains a need to provide a method of producing a focussed immune response specific to one or more particular epitopes. An aim of the present invention

is to provide one or more compositions that can be used to elicit a focussed, and preferably protective, immune response.

According to a first aspect the invention provides an immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, wherein the composition is capable of eliciting an immune response to the first antigen when administered to a human or non-human animal.

The second antigen is preferably generated by mutating the first antigen such that there is at least one different epitope between the first and second antigens. Alternatively the second antigen may be essentially equivalent to the first antigen, for example, an equivalent protein derived from a different but related organism or from an organism carrying a particular mutation, provided the first and second antigens differ in at least one epitope. The second antigen could comprise one or more loop deletions from the first antigen. Alternatively, the second antigen could be identical to the first antigen except that a cluster of amino acids in a single epitope, or in multiple epitopes, on the second antigen may have been changed relative to the first antigen. A cluster of amino acids may comprise at least about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids.

Preferably, where the first and second antigens are proteins or peptides they have at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more sequence identity; preferably at least 75% sequence identity; preferably at least 80% sequence identity; preferably at least 85% sequence identity; preferably at least 90% sequence identity; preferably at least 95% sequence identity.

Preferably if the first and second antigens are large proteins, or are based on large proteins, such as gp120, with at least 100, 200, 300 or more amino acids, the percentage sequence identity between the first and second antigens is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or more; preferably at least 80% sequence identity; preferably at least 85% sequence identity; preferably at least 90% sequence identity; preferably at least 95% sequence identity.

If the first and second antigens are smaller proteins or peptides, and have for example less than about 50, 40, 30, 20 or 15 amino acids, then the percentage sequence identity

between the first and second antigens is preferably at least about 60%, 70%, 75%, 80%, 85% or more; preferably at least 70% sequence identity; preferably at least 75% sequence identity; preferably at least 80% sequence identity. In a peptide of 15 amino acids, 75% sequence identity allows there to be 3 or 4 amino acid changes.

The percent sequence identity is determined by standard alignment algorithms such as, for example, Basic Local Alignment Tool (BLAST) described in Altshul et al. (1990) J. Mol. Biol., 215: 403-410, the algorithm of Needleman et al. (1970) J. Mol. Biol., 48: 444-453, or the algorithm of Meyers et al. (1988) Comput. Appl. Biosci. , 4: 11-17.

Preferably the first and second antigens are both proteins or peptides.

The first antigen may be a wild type protein or peptide, such as a surface protein, or part thereof, from a pathogen. Alternatively the first antigen may be a tumour antigen, or peptide derived thereform, associated with one or more cancers. Alternatively, the first antigen could be a protein or peptide in a transition state and the second antigen may be the same protein or peptide in its native state.

The invention may be used to generate peptide/epitope specific B-cells, either in a human or a non-human animal, which may then be used for the production of therapeutic antibodies, in particular therapeutic monoclonal antibodies. For example, if the first antigen is a tumour antigen, the invention may be used to generate tumour specific B-cells for the production of anti-cancer therapeutic monoclonal antibodies. Preferably antibodies raised in this way display increased sensitivity to ADCC (antibody dependent cellular cytotoxicity) and/or promote an immune response to, for instance, the specific binding surface of a receptor coupling site, and not to the rest of the molecule. This may enhance the 'hit-rate' of useful hybridomas, and/or allow for the production of focussed 'polyclonal' antibody sera directed to a specific region of a protein or peptide.

The invention may also be used to generate catalytic antibodies, in which case the first antigen may be the 'transition state' structure of the antigen, and the second antigen may be the native structure of antigen. This may be used to focus the immune response to the chemical moieties of the transition state groups.

Preferably the first antigen and/or the second antigen comprise more than one epitope.

The first antigen and/or second antigen may independently be isolated from a pathogen or tumour cell, produced recombinantly, or synthetically produced, for example produced by *in vitro* peptide synthesis or *in vitro* translation.

The first and/or second antigen may be a protein derived from a pathogen or disease state, such as cancer, or may be an analogue thereof. An analogue may include a variant in which one or more residues have been added, deleted, inserted or substituted, while having no material effect on the function of the protein. In the case of the second antigen the addition, deletion, insertion or substitution of a residue may be sufficient to alter one or more of the epitopes presented in the folded protein. A residue, or residues, may be added or deleted from one or both end of a naturally occurring protein, or they may be inserted within the protein or substituted for one or more residues within the protein. As would be understood by a person of ordinary skill in the art, one or more protein residues may be added, deleted, inserted or substituted while still maintaining the function and structure of the protein, similarly these changes may or may not alter the immunogenic properties of the antigen. Again, the person of ordinary skill in the art would be able to determine what substitutions or changes would affect the immunogenic properties of the antigen. Furthermore, a conservative substitution of one or more residues within a protein may result in a protein analogue. As would be well understood to the person of ordinary skill in the art, a conservative substitution includes a substitution of one amino acid residue for another having one or more similar chemical properties, such as polarity, charge, hydrophobicity, or aromaticity, for example.

In addition to the immunogenic part the first and/or second antigen may further comprise additional sequence, for example for use in purification of the antigen. For example, if the antigen is a recombinant protein the protein may include a tag, such as a his-tag sequence, for use in protein purification.

The toxin may be any toxin which on internalisation by a B-cell results in cell non-proliferation and/or cell death. Examples of suitable toxins include auristatin, calicheamicin or PE38 (*Pseudomonas* exotoxin 38) domains 2 and 3.

The toxin and second antigen may be linked using any suitable means. For example the toxin may be coupled to streptavidin and the second antigen may be biotinylated, the two may then be linked via the streptavidin biotin interaction. Alternatively, the toxin may be coupled to biotin and the second antigen may be linked to an avidin group, the two may then be linked via the avidin biotin interaction. Alternatively, the antigen may be expressed as a fusion protein with streptavidin, and a biotin-conjugated toxin may be linked via the streptavidin-biotin interaction to the antigen. Alternatively the toxin may be linked directly to the antigen using a specific chemical reaction, for example coupling to the antigen's lysine residues or glycan groups.

In one embodiment, the first antigen may be the HIV-1 gp120 protein, or an immunogenic part thereof. The gp120 protein, or an immunogenic part thereof, may be produced recombinantly. An immunogenic part of the gp120 protein may include any part of the gp120 protein which is capable of eliciting an immune response when administered to a human or non-human animal. The antigen may alternatively comprise an analogue of an immunogenic part of the gp120 protein.

If the first antigen is the HIV-1 gp120 protein, or a part thereof, the second antigen may be a mutated form of HIV-1 gp120, or a part thereof, or a gp120 protein, or a part thereof, from a different viral strain. The mutation may be in the CD4 binding site. Alternatively or additionally the mutation may be in a V-loop and/or in any other neutralisation target. In an embodiment, the second antigen comprises the mutation D368R in the CD4 binding site of the HIV-1 gp120 protein. In a preferred embodiment the mutated gp120 protein is linked to the toxin PE38 domains 2 and 3.

Preferably if the second antigen is a mutated form of the first antigen it has been mutated such as to change at least one epitope in the second antigen compared to the first antigen.

Preferably, upon administration of a composition according to the invention to a human or non-human animal, all B-cells recognising the second antigen are deleted. This deletion preferably arises because upon internalisation of the second antigen the attached toxin causes the death, or at least the non-replication, of the B-cell. Thus, only B-cells responding to one or more epitopes on the first antigen, which are not

present on the second antigen, will remain and be able to proliferate. Preferably the remaining B-cells proliferate and produce neutralising antibodies directed to the epitope or epitopes unique to the first antigen. By designing the unique epitope or epitopes on the first antigen to be epitopes which are known to generate neutralising antibodies the production of neutralising antibodies can be engineered. Preferably, there is only one epitope different between the first and second antigens and the result is a composition which produces neutralising antibodies. The antibodies may be essentially monoclonal and/or polyclonal/oligoclonal. Preferably the antibodies are directed to the epitope in the first antigen which has been mutated in the second antigen.

Preferably the immune response elicited by a composition of the invention is effective against a pathogen from which the first antigen is derived, the immune response may also be effective against a related pathogen.

Preferably the immune response elicited by a composition of the invention affects the ability of a pathogen from which the antigen is derived to infect an immunised human or non-human animal. The immune response elicited may impede or prevent the replication of a pathogen from which the first antigen is derived or a related pathogen. The immune response elicited may recognise and destroy a pathogen from which the first antigen is derived or a related pathogen. The immune response elicited may impede or prevent a pathogen from which the first antigen is derived, or a related pathogen, from causing disease.

In a preferred embodiment the composition produces an antibody response when administered to human or non-human animals.

Preferably the immune response elicited produces substantially epitope-specific antibodies which are preferably substantially neutralising. Preferably the antibodies are directed to an epitope in the first antigen that has been mutated in the second antigen.

Alternatively, the second antigen may be mutated at more than one site, this may result in multiple antibody responses to the non-mutated sites in the first (non-mutated) antigen.

Preferably the immune response elicited in response to the composition of the invention is a neutralising response. Preferably the neutralising response produces antibodies that inhibit or neutralise the biological effect of an antigen, and hence of a pathogen if the antigen is derived from a pathogen. Preferably the antibodies are active against a pathogen or disease state that expresses the first antigen.

The first antigen may be derived from any pathogenic organism, such as a virus, yeast or bacteria. Examples of viruses that the composition of the invention could be used as a vaccine for include, HIV, SIV, H5N1, influenza viruses, Dengue virus, Hepatitis viruses, Herpes viruses, human papilloma virus, pox viruses, polioviruses etc. Examples of bacteria that the composition of the invention could be used as a vaccine for include, *Pseudomonas aeringosa*, *Staphyloccous aureus*, *Neisseria meningitides*, invention could be used to target include HLA-A2-NY-ESO-1, HLA-MAGE-1/3 complexes, VEGF and VEGF receptor.

The invention can be readily applied to any disease antigen where the epitope or epitopes for producing a neutralising response are known or predicted.

In a composition of the invention the first antigen is preferably folded into its native configuration.

A composition of the invention may comprise a further one or more antigens. The further antigens may be derived from the same or a different pathogen, or from the same or different disease state.

The composition of the invention may further comprise an adjuvant, wherein an adjuvant enhances the protective efficacy of the composition. Suitable adjuvants will be well known to those skilled in the art, and may include emulsifiers, muramyl dipeptides, avridine, aqueous adjuvants such as aluminium hydroxide, chitosan-based adjuvants, monophosphoryl lipid A and any of the various saponins, oils and other substances known in the art, such as, Amphigen, LPS, bacterial cell wall extracts, bacterial DNA, CpG sequences, synthetic oligonucleotides, and combinations thereof. Other suitable adjuvants can be formed with an oil component, such as a single oil, a mixture of oils, a water-in-oil emulsion, or an oil-in-water emulsion. The oil may be

mineral oil, vegetable oil, or animal oil. Freund's Complete Adjuvant (FCA) and Freund's Incomplete Adjuvant (FIA) are two common adjuvants used in vaccine compositions, and are also suitable for use in this invention.

The composition may also comprise polymers or other agents to control the consistency of the composition, or to control the release of the protein from the composition.

The composition may also comprise other agents such as diluents, which may include water, saline, glycerol or other suitable alcohols etc; wetting or emulsifying agents; buffering agents; thickening agents for example cellulose or cellulose derivatives; preservatives; detergents; antimicrobial agents; and the like.

Preferably the active ingredients in the composition are greater than 50% pure, usually greater than 80% pure, often greater than 90% pure and more preferably greater than 95%, 98% or 99% pure. With active ingredients approaching 100% pure, for example about 99.5% pure or about 99.9% pure, being used most often.

Preferably, if the composition is for use as a vaccine, the composition comprises an immunologically effective amount of a first and/or a second antigen, preferably of a first antigen. An "immunologically effective amount" of an antigen is an amount that when administered to an individual, either in a single dose or in a series of doses, is effective for treatment or prevention of infection by a pathogen or related pathogen from which the antigen is derived or is effective for the treatment or prevention of a particular disease state. This amount will vary depending upon the health and physical condition of the individual to be treated and on the antigen. Determination of an effective amount of an immunogenic or vaccine composition for administration to an organism is well within the capabilities of those skilled in the art.

A composition according to the invention may be for oral, systemic, parenteral, topical, mucosal, intramuscular, intravenous, intraperitoneal, intradermal, subcutaneous, intranasal, intravaginal, intrarectal, transdermal, sublingual, inhalation or aerosol administration.

The composition may be arranged to be administered as a single dose or as part of a multiple dose schedule. Multiple doses may be administered as a primary immunisation followed by one or more booster immunisations. Suitable timings between priming and boosting immunisations can be routinely determined.

A composition according to the invention may be used in isolation, or it may be combined with one or more other immunogenic or vaccine compositions, and/or with one or more other therapeutic regimens.

Compositions of the invention may be able to induce neutralising antibody responses. These responses are conveniently measured in mice and the results are a standard indicator of vaccine efficacy.

The compositions of the invention may also, or alternatively, be able to elicit an immune response which neutralises bacterial proteins or other molecules, thereby preventing them from having their normal function and preventing or reducing disease progression without necessarily destroying the pathogen.

The composition may be used to elicit/produce a protective immune response when administered to a subject.

The composition may be used as a prophylactic or a therapeutic vaccine directed to a pathogen, or a related pathogen, from which the antigen is derived or to a disease state, for example a cancer.

According to a further aspect, the invention provides a pharmaceutical composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, and a pharmaceutically acceptable carrier or excipient.

Preferably the pharmaceutical composition comprises an immunogenic composition according to the first aspect of the invention.

Preferably the pharmaceutical composition is capable of producing a protective immune response.

Suitable excipients and carriers will be well known to those skilled in the art. These may include solid or liquid carriers. Suitable liquid carriers include water and saline. The antigens of the composition may be formulated into an emulsion or they may be formulated into biodegradable microspheres or liposomes.

According to a further aspect, the present invention provides the use of an immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin in the preparation of a medicament for eliciting an immune response. The medicament may be used for the prophylactic or therapeutic vaccination of subjects against pathogens, or pathogens related to the pathogen, from which the first antigen is derived. The medicament may alternatively be used for the prophylactic or therapeutic vaccination of subjects against a disease state, such as cancer, from which the first antigen is derived. The medicament may be a prophylactic or a therapeutic vaccine.

According a still further aspect, the present invention provides a method of protecting a human or non-human animal from the effects of infection by a pathogen comprising administering to the human or non-human animal a composition according to any other aspect of the invention. The composition may be a vaccine.

According to another aspect, the invention provides a method for raising an immune response in a human or non-human animal comprising administering a pharmaceutical composition according to the invention to the human or non-human animal. The immune response is preferably protective. The method may raise a booster response in a patient that has already been primed. The immune response may be prophylactic or therapeutic.

One way to check the efficacy of a therapeutic treatment comprising administration of a composition according to the invention involves monitoring for pathogen infection after administration of the composition. One way to check the efficacy of a prophylactic treatment comprising administration of a composition according to the invention involves monitoring immune responses to a pathogen from which the antigen is derived after administration of the composition.

According to another aspect, the invention provides the use of an immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, in the preparation of a medicament for use in the immunisation of human or non-human mammals against infection by a pathogen, or related pathogen, from which the first antigen is derived.

According to another aspect, the invention provides the use of an immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, in the preparation of a medicament for use in the immunisation of human or non-human mammals against a disease from which the first antigen is derived.

According to a further aspect, the invention provides a kit for use in inducing an immune response in an organism, comprising an immunogenic or vaccine composition according to the invention and instructions relating to administration.

According to a still further aspect, the invention provides a method of eliciting an immune response in a subject comprising administering a first antigen and a second antigen linked to a toxin to the subject. The first and second antigen may be as described herein. The first antigen and second antigen may be administered in the same or separate compositions. If administered in separate compositions the compositions may be administered simultaneously or sequentially or separately. In an embodiment the subject may be first administered the second antigen linked to a toxin to deplete circulating B-cells with the 'incorrect' specificities, and then subsequently administered either the first antigen alone, or a mix of the first antigen and the second antigen linked to a toxin.

In one embodiment, the invention provides a method of eliciting an immune response in a subject comprising first administering to a subject a second antigen linked to a toxin, and then subsequently administering to the subject a first antigen. Preferably administration of the second antigen linked to the toxin clears the subject of substantially all B cells which recognise the second antigen. Preferably when the first antigen is administered only B cells which recognise epitopes unique to the first antigen, which are not present on the second antigen, are activated. Preferably the first antigen is administered at least 2 hours, preferably at least 4 hours, 8 hours, 12

hours, 24 hours or more after administration of the second antigen and toxin. The first and second antigens may be as described above.

In an alternative embodiment the first and second antigens are administered simultaneously, or substantially simultaneously but in different compositions.

According to yet another aspect, the invention provides a protein-toxin conjugate. The toxin may be as discussed previously, and the protein may be any suitable protein, but preferably as described above with reference to the second antigen. Preferably when administered to a human or animal the protein-toxin conjugate serves to delete all B cells specific to the conjugated protein.

In addition to their potential use as vaccines, compositions according to the invention may be useful as diagnostic reagents and as a measure of the immune competence of a vaccine.

It will be appreciated that optional features applicable to one aspect of the invention can be used in any combination, and in any number. Moreover, they can also be used with any of the other aspects of the invention in any combination and in any number. This includes, but is not limited to, the dependent claims from any claim being used as dependent claims for any other claim in the claims of this application.

Preferred embodiments of the present invention will now be described, merely by way of example, with reference to the following figures and examples.

Figure 1A provides the wild type amino acid sequence of gp120 (Seq ID No: 1).

Figure 1B provides the amino acid sequence of D368R (Seq ID No: 2) – the underlined amino acid indicates where the mutation has occurred.

Figure 1C provides the amino acid sequence of biotinylated D368R gp120 (Seq ID No: 3) – the underlined amino acid indicates where the mutation has occurred, the section underlined and in italics is the biotinylation tag (Avitag).

Figure 2 provides the amino acid sequence of His-streptavidin-PE38 Domains 2 and 3 (Seq ID No:4). The sequence in italics is the streptavidin, the sequence underlined is a linker, and the sequence in bold is PE-38 domains 3 and 3.

Figure 3 illustrates confocal microscopy images of CD4+ cells incubated with the gp120 protein.

PE-38 Domains 2 and 3

PE-38 domains 2 and 3 (Figure 2) can be readily expressed in *E. coli* using well known recombinant technology and purified for use. Examplary protocols for the preparation and purification of PE-38 domains 2 and 3 can be found in Onda et al Journal of Immunology, 2006, 177: 8822-8834 Pastan et al. Methods Mol. Biol., 2004, 248: 503-518.

gp120 PROTEIN AND D368R gp120 PROTEIN

Figure 1A provides the wild type amino acid sequence of mature gp120.

Figure 1B provides the amino acid sequence of D368R gp120, this is a mutant form of Figure 1, in which position 368 has been mutated to arginine.

Figure 1C provides the amino acid sequence of biotinylated D368R gp120

PE38 TOXICITY

Figure 3 illustrates by confocal microscopy that when CD4+ cells are incubated with the gp120 protein, the protein adheres to the cell and then is internalised. The protein in visualised using an anti-His FITC-anti-mouse Fc antibody (the gp120 protein is his-tagged)

To demonstrate the toxicity of PE38, PE38 is complexed to gp120 using the biotin streptavidin interaction and incubated *in vitro* with B-cell lines and clones, CD4 T-cell lines and CD4-transfected Jurkat cells. Cell death is observed when the toxin complex is taken up by the cells.

MOUSE IMMUNISATION STUDIES

To demonstrate the ability of a composition according to the invention to elicit an immune response in mice, Balb/c mice are immunised in the immunisation groups below:

Immunization 1

7 Groups of 4 Balb/c mice per group:	
Group 1	20ug WT gp120 [WT – wildtype]
Group 2	20ug WT gp120, 0.00000002 ug D368R gp120-toxin
Group 3	20ug WT gp120, 0.00002 ug D368R gp120-toxin
Group 4	20ug WT gp120, 0.02 ug D368R gp120-toxin
Group 5	20ug WT gp120, 0.2 ug D368R gp120-toxin
Group 6	20ug WT gp120, 2.0 ug D368R gp120-toxin
Group 7	20ug WT gp120, 20 ug D368R gp120-toxin
Group 8	2.0 ug D368R gp120-toxin
Group 9	20 ug D368R gp120-toxin

The toxin is PE38.

Following immunisation serum samples are obtained and ELISAs are used to determine the specificity of any immune response elicited. The WT gp120 and the D368R mutant gp120 proteins are used as coating antigens in the ELISA.

Groups 8 and 9 in the immunisation, lacking any WT gp120, and containing only antigen linked to toxin, do not generate an immune response as all specific B-cells are deleted.

Group 1 in the immunisation generates a very polyclonal response.

Groups 2-7 in the immunisation generate more CD4 binding site-specific responses, compared to the WT control, and in particular the responses are increasingly focussed, and specific to the WT gp120 CD4 binding site.

Immunization 2

5 Groups of 4 Balb/c mices per group:	
Group 1	2ug OVA
Group 2	2ug OVA, 0.02 ug D368R gp120-toxin
Group 3	2ug OVA, 0. 2 ug D368R gp120-toxin
Group 4	2ug OVA, 2.0 ug D368R gp120-toxin
Group 5	2ug OVA, 20 ug D368R gp120-toxin

The toxin is PE38 domains 2 and 3.

In a second immunization, described above, mice are immunised with OVA (ovalbumin), followed by 2 protein boosts with the D368R gp120-toxin . CAF01 is used as an adjuvant. Again, ELISAs are used to determine the specificities of the immune responses

The results from Groups 1-5 demonstrate that the gp120-toxin component does not block the response to a control antigen (OVA).

Immunization 3

In a third immunisation mice are first administered 10 ug D368R gp120-toxin. This is intended to clear the mouse of substantially all B cells which recognise D368R gp120 The same mice are then immunised 2 to 7 days later with WT gp120, only those B cells specific to the WT region of gp120 mutated in D368R gp120 are stimulated, resulting in a highly specific response.

CLAIMS

1. An immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, wherein the composition is capable of eliciting an immune response to the first antigen when administered to a human or non-human animal.

2. The composition of claim 1 wherein the second antigen is a mutated form of the first antigen.

3. The composition of claim 1 wherein the first and second antigens are equivalent proteins derived from different but related organisms.

4. The composition of any preceding claim wherein the peptides have at least about 75% sequence identity

5. The composition of any preceding claim wherein the first and second antigens are both proteins or peptides.

6. The composition of any preceding claim for use in generating peptide/epitope specific B-cells.

7. The composition of any preceding claim wherein the first and/or second antigens are derived from a pathogen or a disease state

8. The composition of any preceding claim wherein upon internalisation by a B-cell the toxin results in cell non-proliferation and/or cell death.

9. The composition of claim 8 wherein the toxin is selected from the group comprising auristatin, calicheamicin and PE38 (*Pseudomonas* exotoxin 38) domains 2 and 3.

10. A pharmaceutical composition comprising i) a first antigen; and ii) a second antigen linked to a toxin, and a pharmaceutically acceptable carrier or excipient.

11. Use of an immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin in the preparation of a medicament for eliciting an immune response.

12. An immunogenic composition comprising i) a first antigen; and ii) a second antigen linked to a toxin for use in eliciting an immune response.

13. A kit for use in inducing an immune response in an organism, comprising an immunogenic composition according to claim 1 and instructions relating to administration.

14 A method of eliciting an immune response in a subject comprising administering (i) a first antigen and (ii) a second antigen linked to a toxin to the subject.

15. The method of claim 14 wherein the second antigen linked to a toxin is administered before the first antigen is administered.

16. The method of claim 15 wherein the second antigen linked to a toxin is administered at least 2 hours before the first antigen is administered.

SPSTEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNV
WATHACVPTDPNPQEVELENVTENFNMWKNNMVEQMHEDII
SLWDQSLKPCVKLTPLCVTLNCTDLRNATNGNDTNTTSSRE
MMGGGEMKNCSFKITTNIRGKVQKEYALFYELDIVPIDNNSN
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NGKGPCSNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSE
NFADNAKTIIVQLNESVEINCTRPNNNTRKSIHIGPGRALYTTG
EIIGDIRQAHCNLSRAKWNDTLNKIVIKLREQFGNKTIVFKHSS
GGDPEIVTHSFNCGGEFFYCNSTQLFNSTWNVTEESNNTVE
NNTITLPCRIKQIINMWQKVGRAMYAPPIRGQIRCSSNITGLLL
TRDGGPEDNKTEVFRPGGGDMRDNWRSELYKYKVVKIEPL
GVAPTKAISSVVKHHHHHH

Figure 1A

SPSTEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNV
WATHACVPTDPNPQEVELENVTENFNMWKNNMVEQMHEDII
SLWDQSLKPCVKLTPLCVTLNCTDLRNATNGNDTNTTSSRE
MMGGGEMKNCSFKITTNIRGKVQKEYALFYELDIVPIDNNSN
NRYRLISCNTSVITQACPKISFEPIPIHYCAPAGFAILKCKDKKF
NGKGPCSNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSE
NFADNAKTIIVQLNESVEINCTRPNNNTRKSIHIGPGRALYTTG
EIIGDIRQAHCNLSRAKWNDTLNKIVIKLREQFGNKTIVFKHSS
GGRPEIVTHSFNCGGEFFYCNSTQLFNSTWNVTEESNNTVE
NNTITLPCRIKQIINMWQKVGRAMYAPPIRGQIRCSSNITGLLL
TRDGGPEDNKTEVFRPGGGDMRDNWRSELYKYKVVKIEPL
GVAPTKAISSVVKHHHHHH

Figure 1B

SPSTEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVH
NVWATHACVPTDPNPQEVELENVTENFNMWKNNMVEQ
MHEDIISLWDQSLKPCVKLTPLCVTLNCTDLRNATNGNDT
NTTSSSREMMGGGEMKNCSFKITTNIRGKVQKEYALFYE
LDIVPIDNNSNNRYRLISCNTSVITQACPKISFEPIPIHYCA
PAGFAILKCKDKKFNGKGPCSNVSTVQCTHGIRPVVSTQ
LLLNGSLAEEEVVIRSENFADNAKTIIVQLNESVEINCTRP
NNNTRKSIHIGPGRALYTTGEIIGDIRQAHCNLSRAKWND
TLNKIVIKLREQFGNKTIVFKHSSGGRPEIVTHSFNCGGEF
FYCNSTQLFNSTWNVTEESNNTVENNTITLPCRIKQIINM
WQKVGRAMYAPPIRGQIRCSSNITGLLLTRDGGPEDNKT
EVFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAISS
VVGLNDIFEAQKIEWHEGKHHHHHH

Figure 1C

HHHHHH*GDPSKDSKAQVSAAEAGITGTWYNQLGSTFIVT*
AGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGT
ALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWL
LTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAAKKAGVN
*NGNPLDAVQQ*GGGSGGGSGGG**KASGGPEGGSLAALTA**
HQACHLPLETFTRHRQPRGWEQLEQCGYPVQRLVALYL
AARLSWNQVDQVIANALASPGSGGDLGEAIRESPEQARL
ALTLAAAESERFVRQGTGNDEAGAANGPADSGDALLER
NYPTGAEFLGDGGAVSFSTRGTQNWTVERLLQAHRQLE
EGGYVFVGYHGTFLEAAQSIVFGGVRARSQDLDAIWAGF
YIAGDPALAYGYAQDQEPDAAGRIRNGALLRVYVPRSSL
PGFYATSLTLAAPEAAGEVERLIGHPLPLRLDAITGPEES
GGRLETILGWPLAERTVVIPSAIPTDPRNVGGDLDPSSIPD
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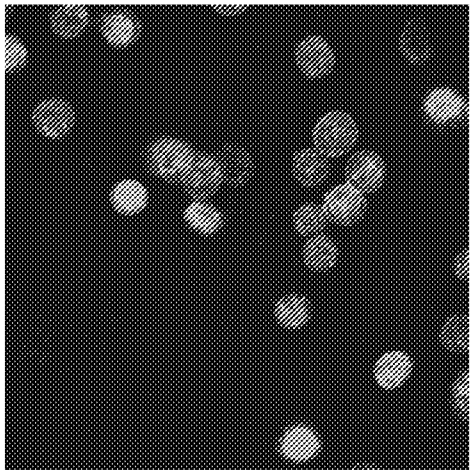
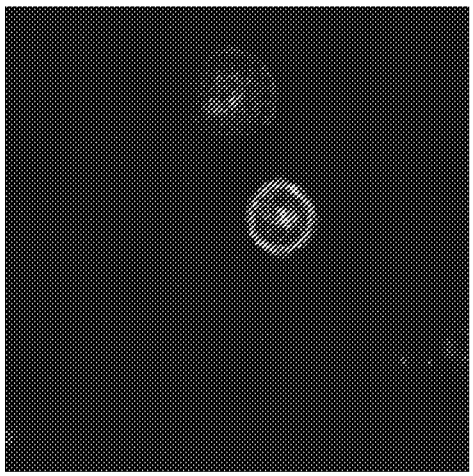
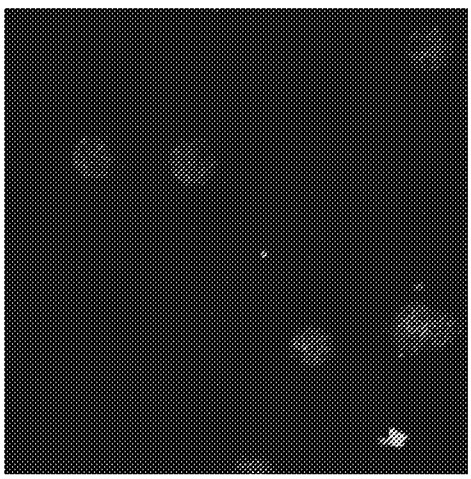
Figure 2



Human CD4+ T-cells



jurkat-hCD4+



jurkat

Figure 3