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(54) Title: IMMUNOSTIMULATORY COMPOSITIONS AND USES THEREFOR

(57) Abstract: Disclosed are compositions and methods for stimulating immune responses. More particularly, these compositions and methods involve the use of an inhibitor of IL-25 function and an immune stimulator that stimulates an immune response to a target antigen for stimulating protective or therapeutic immune responses to a target antigen. The compositions and methods of the present invention are particularly useful in the prevention and treatment of infections and cancers.

TITLE OF THE INVENTION

"IMMUNOSTIMULATORY COMPOSITIONS AND USES THEREFOR"

[0001] This application claims priority to Australian Provisional Application No. 2016902939 entitled "Immunostimulatory Compositions and Uses Therefor" filed 26 July 2016, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates generally to compositions and methods for stimulating immune responses. More particularly, the present invention relates to the co-expression, co-location or co-presentation on host cells (*e.g.* antigen-presenting cells, leukocytes, *etc.*) of an inhibitor of IL-25 function and an immune stimulator that stimulates an immune response to a target antigen in compositions and methods for stimulating protective or therapeutic immune responses to the target antigen. The compositions and methods of the present invention are particularly useful in the prevention and treatment of infections and cancers.

BACKGROUND OF THE INVENTION

[0003] Most infectious agents penetrate the defense barriers of a host at the mucosal membranes or surfaces. Perhaps unsurprisingly, it is becoming increasingly clear that local mucosal immune responses are particularly important for protection against disease (*see*, Levine, M.M., *J Pediatr Gastroenterol Nutr*, 2000, 31: 336-355). In this regard, mucosal immune responses are most efficiently induced by the administration of vaccines onto mucosal surfaces, whereas injected (*i.e.*, systemic) immunizations are generally poor inducers of mucosal immunity and are therefore considered to be less effective against infection at a mucosal surface (*see*, Lamm, M.E., *Annu Rev Microbiol*, 1997, 51: 311-340). Nevertheless, clinical vaccine research continues to remain primarily based on the injection of antigens, administered either intramuscularly or subcutaneously (*see*, Neutra, M.R. and Kozlowski, P.A., *Nat Rev Immunol*, 2006, 6: 148-158).

[0004] For many pathogens, optimal protection is likely to require in parallel both mucosal and systemic immune responses. The most effective mucosal vaccine strategies might be prime-boost combinations that involve both mucosal and systemic delivery. It has been reported that mucosal immunization can prime the immune system for both systemic and mucosal responses, presumably by inducing the expression of both mucosal and systemic homing receptors by responding lymphocytes (*see*, Kunkel, E.J., and Butcher, E.C., *Nature Rev Immunol*, 2003, 3: 822-829). By contrast, parenteral priming is unlikely to prime the immune system for subsequent mucosal vaccination (Mestecky, J., *J Clin Immunol*, 1987, 7: 265-276).

[0005] It has previously been established that mucosal immunizations can generate high-avidity HIV-specific $CD8^+$ T cells, as compared to systemic immunization. The present inventors have demonstrated that the cytokine interleukin (IL)-13 is detrimental to the functional avidity of these T cells, and that T cell avidity is significantly improved by transient inhibition of IL-13 function in the local milieu of the immune response (*see*, Ranasinghe *et al.*, *Mucosal Immunity*, 2013, 6(6): 1068-1080).

[0006] Although many of the current HIV vaccine trials show enhanced immunity in animals, they have failed to establish true correlates of protection. These findings increasingly suggest that not only the magnitude but also the "quality" or "avidity" of the T cell response

generated against vaccine antigens may be important in protection against pathogenic organisms such as HIV-1. The quality of the T cell response is reflected in the functional avidity of T cells towards the MHC-peptide complex on target cells. High avidity cytotoxic T lymphocytes (CTL) recognise low concentrations of antigen, whilst low avidity CTL are functionally ineffective at these antigen concentrations (Alexander-Miller *et al.*, 1996, *J. Exp. Med.* 184: 485-492; La Gruta *et al.*, 2004, *J. Immunol.* 172: 5553-5560). It is now well established that high avidity CTL also have greater capacity to clear an infection compared to low avidity T cells (Alexander-Miller *et al.*, 2005, *Immunol. Res.* **31**: 13-24).

[0007] Moreover, local antiviral immune responses in the genital and rectal tissues where HIV is usually first encountered are vital. Local immune responses in the gastro-intestinal tract are also important given that it is a major site of HIV replication. The presence of high-avidity antiviral T cells at sites of initial HIV exposure, *i.e.*, the mucosa, offers great potential for reducing mucosal $CD4^+$ T cell depletion and local control of HIV infection. Given their demonstrated capacity to recognise target cells expressing very low levels of viral antigen (*e.g.*, early after infection of a cell), and a more rapid initiation of target cell lysis even at low concentrations of target antigen, vaccine strategies that elicit high-avidity CTL are likely to offer substantial protection against infection.

[0008] The innate lymphoid cells (ILC) are a recently identified class of lymphocytes that do not express antigen receptors (in contrast to T or B cells), or surface markers characteristic of other immune cell types, *i.e.*, Lineage negative (Lin^-), (*see*, Artis, D., and Spits, H., *Nature*, 2015, 517(7534): 293-301).

[0009] ILC are currently characterized into three sub-groups based upon their cell surface receptors, transcription factors and cytokine expression. Group 1 ILC ("ILC1") are T-bet^+, and express IFN-γ and TNF-α. Group 2 ILC ("ILC2") are IL-$25R^+$, IL-$33R^+$, $TSLPR^+$, and $GATA3^+$, and express cytokines IL-5, IL-13, IL-9 and IL-4. Group 3 ILC ("ILC3") are $ROR\gamma t^+$ and express cytokines IL-17A and IL-22. Notably, recent publications suggest that ILC type may be fluid depending upon the cytokine environment determining ILC differentiation. For example, ILC3 and ILC2 can reportedly develop into ILC1 when activated by either IL-12 or IL-18 (*see*, Bernink, J.H., *et al.*, *Immunity*, 2015, 43(1): 146-60; and Lim, A.I., *et al.*, *J Exp Med*, 2016).

[0010] The majority of ILC studies concern inflammatory conditions such as allergy, asthma and atopic dermatitis (ILC2), psoriasis and inflammatory bowel disease (ILC1 and ILC3). ILC2 cells have been shown to be required for Th2 mediated immunity towards parasitic helminth infections (*see*, Artis and Spits, *supra*). The requirement of ILC1 in Th1 mediated immunity towards intracellular infections (*e.g.*, viruses) and ILC3 in Th17 mediated immunity towards bacterial and fungal infections has been proposed. The specific molecular mechanisms by which these cell types might act is yet to be fully understood.

SUMMARY OF THE INVENTION

[0011] The present inventors have surprisingly discovered that the avidity of antigen-specific immune responses to a target antigen can be increased by inhibiting IL-25 function. This co-administration (*i.e.*, antigen and inhibitor of IL-25 function) has particular advantages in the prevention or treatment of any diseases and/or conditions that are associated with the presence or aberrant expression of a target antigen in a subject.

[0012] Thus, in one aspect, the present invention provides immunostimulatory compositions for stimulating an immune response to a target antigen in a subject. In certain embodiments, the immune response is a T-cell mediated response. In another aspect, the present invention provides compositions for preventing or treating a disease or condition associated with the presence or aberrant expression of a target antigen in a subject.

[0013] The immunostimulatory compositions of the present invention generally comprise a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject, together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible.

[0014] In some embodiments, the immunostimulatory composition comprises a nucleic acid composition comprising: a first agent comprising a coding sequence for an immune stimulator operably linked to a regulatory polynucleotide, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject and a second agent comprising a coding sequence for an inhibitor of IL-25 operably linked to a regulatory polynucleotide.

[0015] In some embodiments, the immune stimulator is selected from an antigen that corresponds to at least a portion of the target antigen. The target antigen is typically associated with a disease or condition of interest, including but not limited to pathogenic infections and cancers, such as but not limited to human immunodeficiency virus (HIV) infection, tuberculosis (TB), non-pharyngeal carcinoma, and hepatitis C, as well as agricultural diseases such as bovine tuberculosis and Johne's disease. The antigen that corresponds to at least a portion of the target antigen may be provided to the subject in soluble form (*e.g.*, a peptide or polypeptide), or in soluble form when expressed.

[0016] In some embodiments, the inhibitor of IL-25 function is selected from a variant form of IL-25, soluble or defective IL-25 receptors or fragments thereof, or antigen-binding molecules that are immuno-interactive with IL-25 or an IL-25 receptor.

[0017] In some embodiments, the subject is naïve to the target antigen or has previously raised an immune response to the target antigen. Suitably, in embodiments in which the subject has previously raised an immune response to the target antigen and the immune stimulator comprises an antigen that corresponds to the target antigen, the amino acid sequence of the corresponding antigen is the same as the amino acid sequence of at least a portion of the target antigen. In illustrative examples of this type, the corresponding antigen is a naturally-occurring antigen to which the subject has previously raised an immune response.

[0018] Exemplary immunostimulatory compositions of the present invention include vaccines or constructs, including but not limited to recombinant vaccines.

[0019] Notably, the immunostimulatory compositions of the present invention have any one or more activities selected from the group consisting of: stimulating or inducing the development of a Th17 response to the antigen; stimulating or inducing the development of a Th1 response to the antigen; suppressing the expression of any one or more of IL-13, IL-4 IL-9, and IL-5 by ILC2 cells; stimulating the development of ILC1; and stimulating the development of ILC3;

[0020] In some embodiments, the immunostimulatory compositions further comprises one or more ancillary agents. For example, a suitable ancillary agent is a cytokine selected from the group consisting of IL-12, IL-3, IL-5, TNF-α, GMCSF, and IFN-γ.

[0021] In some embodiments, the immunostimulatory compositions further comprise a pharmaceutically acceptable carrier or diluent. In some embodiments, the compositions further comprise an adjuvant that enhances the effectiveness of the immune stimulation. Suitably, the adjuvant delivers the antigen to the class I major histocompatibility (MHC) pathway. For example, such adjuvants include, but are not limited to, saponin-containing compounds (*e.g.*, ISCOMs) and cytolysins, which mediate delivery of antigens to the cytosol of a target cell. The cytolysin may be linked to, or otherwise associated with, the antigen. In some embodiments, the cytolysin mediates transfer of the antigens from the vacuole (*e.g.*, phagosome or endosome) to the cytosol of an antigen-presenting cell and in illustrative examples of this type, the cytolysin is a listeriolysin.

[0022] In some embodiments, the antigen comprises, or is otherwise associated with, an intracellular degradation signal or degron. In illustrative examples of this type, the intracellular degradation signal comprises a destabilizing amino acid at the amino-terminus of the antigen. Suitably, the destabilizing amino acid is selected from isoleucine and glutamic acid, preferably from histidine tyrosine and glutamine, and even more preferably from aspartic acid, asparagine, phenylalanine, leucine, tryptophan and lysine. In a specific embodiment, the destabilizing amino acid is arginine. In other illustrative examples of this type, the antigen is fused or otherwise conjugated to a masking entity, which masks the amino terminus so that when unmasked the antigen will exhibit an enhanced rate of intracellular proteolytic degradation. Suitably, the masking entity is a masking protein sequence. The masking protein sequence is suitably cleavable by an endoprotease, which is typically an endogenous endoprotease of a mammalian cell. For example, an endoprotease cleavage site may be interposed between the masking protein sequence and the antigen. Suitable endoproteases include, but are not restricted to, serine endoproteases (*e.g.*, subtilisins and furins), proteasomal endopeptidases, proteases relating to the MHC class I processing pathway and signal peptidases. In a preferred embodiment of this type, the masking protein sequence comprises a signal peptide sequence. Suitable signal peptides sequences are described, for example, by Nothwehr *et al.* (1990,. *Bioessays* 12 (10): 479-484); Izard, *et al.* (1994, *Mol. Microbiol.* 13 (5): 765-773); Menne, *et al.* (2000, *Bioinformatics.* 16 (8): 741-742); and Ladunga (2000, *Curr. Opin. Biotechnol.* 11 (1): 13-18).

[0023] Alternatively, or in addition, the intracellular degradation signal comprises a ubiquitin acceptor, which allows for the attachment of ubiquitin by intracellular enzymes, which target the antigen for degradation *via* the ubiquitin-proteosome pathway. Suitably, the ubiquitin acceptor is a molecule which contains a residue appropriately positioned from the amino terminus of the antigen as to be able to be bound by ubiquitin molecules. Such residues may have an epsilon amino group such as lysine. In illustrative examples of this type, the ubiquitin acceptor comprises at least one, preferably at least two, more preferably at least four and still more preferably at least six lysine residues, which are suitably present in a sufficiently segmentally mobile region of the antigen.

[0024] In some embodiments, the intracellular degradation signal comprises a ubiquitin or biologically active fragment thereof. In non-limiting examples of this type, the ubiquitin or biologically active fragment thereof is fused, or otherwise conjugated, to the antigen. Suitably, the

ubiquitin is of mammalian origin. By way of an illustrative example, a suitable ubiquitin could be of human or other primate origin.

[0025] Another aspect of the present invention provides methods for stimulating an immune response to a target antigen in a subject. In certain embodiments, the immune response is a T cell mediated response (*e.g.*, a $CD8^+$ T cell response, and/or a $CD4^+$ T cell response). In some of the same and other embodiments, the immune response is a B cell mediated response. A further aspect of the present invention provides methods for treating or preventing a disease or condition associated with the presence or aberrant expression of a target antigen in a subject.

[0026] The methods of the present invention generally comprise administering to the subject an effective amount of a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible, as broadly described above. The target antigen is typically associated with a disease or condition of interest, including but not limited to pathogenic infections and cancers, such as but not limited to HIV, TB, non-pharyngeal carcinoma, hepatitis C, as well as agricultural diseases such as bovine tuberculosis and Johne's disease.

[0027] In some embodiments, the subject is naïve to the target antigen or has previously raised an immune response to the target antigen. Suitably, in embodiments in which the subject has previously raised an immune response to the target antigen and the immune stimulator comprises an antigen that corresponds to the target antigen, the amino acid sequence of the corresponding antigen is the same as the amino acid sequence of at least a portion of the target antigen. In illustrative examples of this type, the corresponding antigen is a naturally-occurring antigen to which the subject has previously raised an immune response.

[0028] In some embodiments, the first agent and the second agent are administered intramuscularly. It is proposed that the inhibitor of IL-25 function may sequester the availability of free IL-25 to bind muscle cell IL-25R and thus provide a transient blockade of IL-25 signaling.

[0029] In yet another aspect, the invention contemplates the use of first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible as broadly defined above in the manufacture of a medicament for stimulating an immune response to the target antigen in a subject. In certain embodiments, the immune response is a T cell mediated response (*e.g.*, a $CD8^+$ T cell response, and/or a $CD4^+$ T cell response). The target antigen is typically associated with a disease or condition of interest, including but not limited to pathogenic infections and cancers, such as, but not limited to HIV, TB, non-pharyngeal carcinoma, and hepatitis C. Agricultural diseases such as bovine tuberculosis and Johne's disease are also contemplated.

[0030] In still another aspect, the invention resides in the use of a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence

encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible as broadly defined above in the manufacture of a medicament for preventing or treating a disease or condition associated with the presence or aberrant expression of the target antigen in a subject. The target antigen is typically associated with a disease or condition of interest, including but not limited to pathogenic infections and cancers, such as but not limited to HIV, TB, non-pharyngeal carcinoma and hepatitis C, as well as agricultural diseases such as bovine tuberculosis and Johne's disease.

[0031] In one aspect, the present invention provides a method of enhancing a Th17 immune response in a subject, wherein the Th17 immune response can be determined by detecting an increase in the expression of any one of the cytokines: IL-17A, IL-22, TNF-α, IL-17F, IL-21, IL-26, GM-CSF, and MIP-A.

[0032] In various aspects, the present invention is directed to a method of enhancing a Th1 immune response, wherein the Th1 immune response comprises an increase in the expression of any one of IFN-γ, IL-2 and TNF-β.

[0033] In yet some other aspects, the present invention provides methods for suppressing the expression of one or more cytokines selected from any one of IL-13, IL-4, IL-9, and IL-5 at the vaccination site of the subject.

[0034] Yet another aspect of the present invention provides an immunostimulatory antigen-presenting cell or antigen-presenting cell precursor that presents an antigen that corresponds to at least a portion of the target antigen, and wherein the antigen-presenting cell or antigen-presenting cell precursor expresses or otherwise produces an inhibitor of IL-25 function.

[0035] Yet a further aspect of the present invention provides a method for producing an immunostimulatory antigen-presenting cell, the method comprising contacting an antigen-presenting cell or antigen-presenting cell precursor with an antigen that corresponds to at least a portion of the target antigen or a composition of the invention for a time and under conditions sufficient for the antigen or a processed form thereof to be presented by the antigen-presenting cell or antigen-presenting cell precursor, and wherein the antigen-presenting cell or antigen-presenting cell precursor expresses or otherwise produces an inhibitor of IL-25 function.

[0036] Still another aspect of the present invention provides a method for enhancing mucosal immunity to a target antigen in a subject that is administered a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to the target antigen, the method comprising systemically administering to the subject a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible, as broadly described above, to thereby enhance mucosal immunity to the target antigen. In certain embodiments, the first agent and the second agent are systemically administered concurrently to the subject.

[0037] In a related aspect, the present invention provides a use of an IL-25 inhibitory agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide

sequence encoding an inhibitor of IL-25 function is expressible, as broadly described above, for enhancing mucosal immunity to a target antigen by an immunostimulatory agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to the target antigen, wherein the IL-25 inhibitory agent is formulated for systemic administration. In some embodiments, both the IL-25 inhibitory agent and the immunostimulatory agent are formulated for concurrent administration via a systemic route. In specific embodiments, the systemic route is intramuscular.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] Figure 1 shows a vector map of the (A) FPV vector pAF09-IL25BP, and (B) MVA vector pMVAdIII-IL25BP.

[0039] Figure 2. Evaluation of ILC2 following FPV-HIV vaccination. Following intramuscular (i.m.) vaccination with FPV-HIV, $CD45^+$ cells were analyzed by flow cytometry for lineage negative cells. FITC-conjugated anti-mouse CD3 (clone 17A2), CD19 (clone 6D5), CD11b (clone M1/70), CD11c (clone N418), CD49 (clone HMα2), FcεRIα (clone MAR-1) (all lineage positive markers were selected as FITC) were used to gate out the lineage-positive cells containing, T cells ($CD3^+$), B cells ($CD19^+$), macrophages and dendritic cells ($CD11b^+$, $CD11c^+$), NK cells ($CD49^+$), mast cells and basophils ($FcεRIα^+$) The Lineage negative population (A, bottom left quadrant) was further analyzed for ST2 (IL-33R) . Data indicated that in ILC2 in muscle do not express IL-33R but express IL-25R (B). When $IL\text{-}25R^+$ ILC2 were further characterized for IL-13 (C) and IL-4 (D) expression by intracellular cytokine staining, data revealed that activated muscle ILC2 cells express IL-13 but not IL-4.

[0040] Figure 3. Evaluation of ILC 1 & 3 following unadjuvanted FPV-HIV vaccination. Following FPV-HIV vaccination, muscle ILC that were $CD45^+$, Lin^-, $IL\text{-}33R^-$ (as shown in A) were further analyzed for cell surface $NKp46^-$ and $NKp46^+$ (B) innate lymphoid cells containing ILC1 and ILC3 cell populations.

[0041] Figure 4. Evaluation of ILC1 & ILC3 in muscle following i.m. FPV-HIV vaccination. The 2-D plots A, B, and C indicate the expression pattern of (A) IFN-γ, (B) IL-22 and (C) IL-17A by $CD45^+$ Lin^- $IL\text{-}33R^-$ $NKp46^+$ cells. The 2-D plots D, E, and F indicate the expression pattern of expressing (D) IFN-γ, (E) IL-22 and (F) IL-17A in $CD45^+$ Lin^- $IL\text{-}33R^-$ $NKp46^-$ cells.

[0042] Figure 5. Evaluation of ILC2 following FPV-HIV-IL25RBP i.m. vaccination. BALB/c mice (n = 4) were immunized by intramuscular FPV-HIV-IL25BP and ILC2 evaluated 24 hours post vaccination. Data indicate that following FPV-IL25BP vaccination muscle ILC2 do not express IL-33R ($CD45^+$ Lin^- $IL\text{-}33R^-$ ($ST2^-$)) but in contrast all ILC2 induced (99%) were $IL\text{-}25R^+$ compared to control unadjuvanted FPV-HIV vaccine strategy where only ~ 6% of the ILC2 were $IL\text{-}25R^+$ (*see*, Figure 1). Intracellular cytokine staining indicated that these cells did not express IL-13 or IL-4. Data indicate that transient inhibition of IL-25 activation in muscle by FPV-IL25BP vaccination, completely inhibits IL-13 production by $CD45^+$ Lin^- $IL\text{-}33R^-$ $IL\text{-}25R^+$ ILC2 cells compared to unadjuvanted i.m. FPV–HIV vaccination.

[0043] Figure 6. Evaluation of ILC 1 & 3 following FPV-HIV i.m. vaccination. BALB/c mice (n = 4) were immunized by intramuscular FPV-IL25BP and ILC1-like and ILC3-like cells were evaluated 24 hours post vaccination. Data indicate that following FPV-IL-25BP vaccination a

majority of the $CD45^+$ Lin^- IL-33R^- ILC3 like cells were $NKp46^-$ and expressed elevated IL-17A (~ 98%) and IL-22 (~ 98%), but no or little IFN-γ was detected. Furthermore, no appreciable $NKp46^+$ population (< 1.5%) was detected compared with the non-adjuvanted i.m. FPV-HIV vaccination (~ 86%) (*see*, Figures 2 and 3).

[0044] Figure 7. Evaluation of systemic HIV-specific $CD8^+$ T cell responses following FPV-HIV/MVA-HIV-IL-25RBP adjuvanted vaccination. HIV-specific $CD8^+$ T cell responses following FPV-HIV intranasal (i.n.) prime, followed by MVA-HIVgag-IL-25BP adjuvant intramuscular (i.m.) booster vaccination measured by Kd-Gag-specific tetramer staining in spleen (systemic). The plots represent cells gated on $CD8^+$ T cells.

[0045] Figure 8. Evaluation of mucosal HIV-specific $CD8^+$ T cell responses following FPV-HIV/MVA-HIV-IL-25RBP adjuvanted vaccination. HIV-specific $CD8^+$ T cell responses following FPV-HIV intranasal (i.n.) prime, followed by MVA-HIVgag-IL-25BP adjuvant intramuscular (i.m.) booster vaccination measured by Kd-Gag-specific tetramer staining in Payer's Patch (mucosal). Significantly elevated numbers of HIV-gag specific $CD8^+$ cells were detected in Peyer's Patch with the adjuvanted vaccine strategy compared to the control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV prime-boost vaccine strategy. The plots represent cells gated on $CD8^+$ T cells.

[0046] Figure 9. Evaluation IFN-γ expression by HIVgag-specific $CD8^+$ T cells in spleen (systemic) following i.n. FPV-HIV prime, followed by i.m. MVA-HIVgag-IL-25BP adjuvant booster vaccination. Elevated numbers of HIV-gag specific $CD8^+$ cells expressing IFN-γ were detected in spleen relative to the control unadjuvanted i.n. FPV-HIV/i.m. MVA-HIV vaccination strategy. Note: The plots represent cells gated on $CD8^+$ T cells.

[0047] Figure 10. Evaluation IFN-γ expression by HIVgag-specific $CD8^+$ T cells in Payer's Patches (mucosal) following i.n. FPV-HIV prime, followed by i.m. MVA-HIVgag-IL-25BP adjuvanted booster vaccination. Elevated numbers of HIV-gag-specific $CD8^+$ cells expressing IFN-γ were detected in Peyer's Patch relative to the control unadjuvanted i.n. FPV-HIV/i.m. MVA-HIV vaccination strategy. Note: The plots represent cells gated on $CD8^+$ T cells.

[0048] Figure 11. Evaluation TNF-α expression by HIVgag-specific systemic (spleen; A, B) and mucosal (Peyer's Patch; C, D) $CD8^+$ T cells following i.n. FPV-HIV prime, followed by i.m. MVA-HIVgag-IL-25BP adjuvanted booster vaccination. Although no differences were detected in the mucosal compartment, elevated numbers of TNF-α expressing HIV-gag specific $CD8^+$ cells were detected both in spleen and Peyer's Patch relative to the control unadjuvanted i.n. FPV-HIV/i.m. MVA-HIV vaccination strategy (E). The plots represent cells gated on $CD8^+$ T cells.

[0049] Figure 12. Evaluation polyfunctional (IFN-γ and TNF-α) HIVgag-specific systemic (spleen; A, B) and mucosal (Peyer's Patch; C, D) $CD8^+$ T cells following i.n. FPV-HIV prime, followed by i.m. MVA-HIVgag-IL-25BP adjuvanted booster vaccination. Although no differences were detected in the systemic compartment, elevated numbers of polyfunctional HIV-gag specific $CD8^+$ cells were detected in Peyer's Patch relative to the control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV vaccination strategy (E). The plots represent cells gated on $CD8^+$ IFN-γ^+ T cells.

[0050] Figure 13. Evaluation of IL-22 (A) and IL-17 (C) expressing HIVgag-specific systemic (spleen) $CD8^+$ T cells following i.n. FPV-HIV prime, followed by i.m. MVA-HIVgag-IL-25BP adjuvant booster vaccination. Although low compared to IFN-γ or TNF-α expression, elevated numbers of HIV-gag specific $CD8^+$ T cells that expressed IL-22 (A) and IL-17 (C) were detected in

spleen (E) relative to the control unadjuvanted i.n. FPV-HIV/i.m. MVA-HIV vaccination strategy (unadjuvanted control data shown in B, D). The plots represent cells gated on $CD8^+$ T cells.

[0051] Figure 14. Evaluation of IL-22 (A) and IL-17 (C) expressing HIVgag-specific mucosal (Peyer's Patch) $CD8^+$ T cells following i.n. FPV-HIV prime, followed by MVA-HIVgag-IL-25BP adjuvanted i.m. booster vaccination. Although low compared to IFN-γ or TNF-α expression, elevated numbers of HIV-gag specific $CD8^+$ T cells that expressed of IL-22 (A) and IL-17-γ (C) were detected Peyer's Patches (E) relative to the control unadjuvanted i.n. FPV-HIV/i.m. MVA-HIV vaccination strategy (unadjuvanted control data shown in B, D). Note: The plots represent cells gated on $CD8^+$ T cells.

[0052] Figure 15. Evaluation of cytokine expression by $CD4^+$ and $CD8^+$ T cells, following IL-25BP vaccination. WT BALB/c mice (n=5) were prime-boost immunized two weeks apart using intranasal/ intramuscular vaccine strategy with; (1) FPV/MVA, (2) FPV-IL-25R/MVA-IL-25R and (3) FPV-IL-4R antagonist/ MVA-IL-25R, expressing TB antigens and the expression of IFN-γ, IL-2, IL-17A and TNF-α by $CD4^+$ (top graph) and $CD8^+$ (bottom graph) T cells were evaluated 14 days post booster vaccination.

[0053] Figure 16. Evaluation of lung $lineage^-$ ILC2 subsets 24h post intranasal IL-25BP adjuvanted vaccination. WT BALB/c mice (n = 6) were immunized intranasally with unadjuvanted or IL-25BP adjuvanted FPV vaccines and different ILC2 subsets were evaluated 24h post vaccination. Graphs represents number of (a) $lineage^-$ IL-33R/$ST2^+$ ILC2s. (b) $lineage^-$ IL-33R/$ST2^-$ $IL\text{-}25R^+$ ILC2s (**c**) $lineage^-$ IL-33R/$ST2^-$ $TSLPR^+$ ILC2s back calculated to the $CD45^+$ population 24h post vaccination. The error bars represent the mean and standard deviation (s.d.). The p-values were calculated using GraphPad Prism software (version 6.05 for Windows). * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

[0054] Figure 17. Expression of IL-13 and IL-4 by different ILC2 subsets 24h post intranasal IL-25BP adjuvanted vaccination. The three different ILC2 subsets were further analysed for IL-13 and IL-4 expression 24h post vaccination. The graphs represent (a) IL-13 and (b) IL-4 expression by the different ILC2 subsets. The error bars represent the mean and standard deviation (s.d.). The p-values were calculated using GraphPad Prism software (version 6.05 for Windows). * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

[0055] Figure 18. Expression of IL-13 and IL-4 by $lineage^-$ IL-33R/$ST2^-$ $IL\text{-}25R^-$ $TSLPR^-$ (novel) ILC2 subset 24h post intranasal IL-25BP adjuvanted FPV vaccination. 24h post intranasal IL-25BP adjuvanted FPV vaccination, the expression of IL-4 and IL-13 by different ILC2 subsets were assessed. The FACS plots represent (a) $lineage^-$ IL-33R/$ST2^+$ ILC2, (b) $lineage^-$ IL-33R/$ST2^-$ $IL\text{-}25R^+$ ILC2 (c) $lineage^-$ IL-33R/$ST2^-$ $TSLPR^+$ ILC2 and (d) the novel $lineage^-$ IL-33R/$ST2^-$ IL-25R-$TSLPR^-$ ILC2 expressing IL-13 and IL-4 (bottom right plot).

[0056] Figure 19. Identification of lung $lineage^-$ IL-33R/$ST2^-$ $NKp46^{+/-}$ ILC (ILC1&3) subsets 24h post intranasal IL-25BP adjuvanted vaccination. WT BALB/c mice (n = 6) were immunized intranasally with unadjuvanted and IL-25BP adjuvanted FPV vaccines. The graphs represent number of (a) $lineage^-$ IL-33R/$ST2^-$ $NKp46^+$ ILC and (b) $lineage^-$ IL-33R/$ST2^-$ $NKp46^-$ ILC back calculated to $CD45^+$ cells, 24hpost vaccination. The error bars represent the mean and standard deviation (s.d.). The p-values were calculated using GraphPad Prism software (version 6.05 for Windows). * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

[0057] Figure 20. Evaluation of IFN-γ, IL-22 and IL-17A expression by lineage$^-$ IL-33R/ST2$^-$ NKp46$^+$ and NKp46$^-$ ILC following intranasally IL-25BP adjuvanted FPV vaccination. The graphs indicate the number of NKp46$^+$ and NKp46$^-$ ILC expressing (a and d) IFN-γ, (b and e) IL-22 and (c and f) IL-17A respectively, 24h post unadjuvanted and IL-25BP adjuvanted FPV vaccination. The error bars represent the mean and standard deviation (s.d.). The p-values were calculated using GraphPad Prism software (version 6.05 for Windows). * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0058] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0059] The articles "*a*" and "*an*" are used herein to refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, "*an element*" means one element or more than one element.

[0060] The term "*about*" is used herein to refer to conditions (*e.g.*, amounts, concentrations, time *etc.*) that vary by as much as 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% to a specified condition.

[0061] The terms "*administration concurrently*" or "*administering concurrently*" or "*co-administering*" and the like refer to the administration of a single composition containing two or more actives, or the administration of each active as separate compositions and/or delivered by separate routes either contemporaneously or simultaneously or sequentially within a short enough period of time that the effective result is equivalent to that obtained when all such actives are administered as a single composition. By "*simultaneously*" is meant that the active agents are administered at substantially the same time, and desirably together in the same formulation. By "*contemporaneously*" it is meant that the active agents are administered closely in time, *e.g.*, one agent is administered within from about 1 min to within about 1 d before or after another. Any contemporaneous time is useful. However, it will often be the case that when not administered simultaneously, the agents will be administered within about 1 min to within about 8 h and preferably within less than about 1 h to about 4 h. When administered contemporaneously, the agents are suitably administered at the same site on the subject. The term "same site" includes the exact location, but can be within about 0.5 cm to about 15 cm, preferably from within about 0.5 cm to about 5 cm. The term "*separately*" as used herein means that the agents are administered at an interval, for example at an interval of about a day to several weeks or months. The active agents may be administered in either order. The term "*sequentially*" as used herein means that the agents are administered in sequence, for example at an interval or intervals of minutes, hours, days or weeks. If appropriate the active agents may be administered in a regular repeating cycle.

[0062] As used herein, "*and/or*" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0063] The terms "*antagonist*" and "*inhibitor*" are used interchangeably herein to refer to any molecule that partially or fully blocks, inhibits, stops, diminishes, reduces, impedes, impairs or neutralizes one or more biological activities or functions of IL-25 or a receptor to which it binds (*e.g.*, IL-25R) in any setting including, *in vitro*, *in situ*, or *in vivo*. Likewise, the terms "antagonize", "antagonizing", "inhibit", "inhibiting" and the like are used interchangeably herein to refer to blocking, inhibiting stopping, diminishing, reducing, impeding, impairing or neutralizing an activity or function as described for example above and elsewhere herein. By way of example, the terms "antagonize" and "inhibit" can refer to a decrease of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% in an activity, or function.

[0064] As used herein, the term "*antigen*" and its grammatically equivalents expressions (*e.g.*, "*antigenic*") refer to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (*e.g.*, oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (*e.g.*, polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, toxins, and other miscellaneous antigens.

[0065] By "*antigen-binding molecule*" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity. Representative antigen-binding molecules that are useful in the practice of the present invention include polyclonal and monoclonal antibodies as well as their fragments (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding/recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Antigen-binding molecules also encompass dimeric antibodies, as well as multivalent forms of antibodies. In some embodiments, the antigen-binding molecules are chimeric antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, for example, US Pat. No. 4,816,567; and Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:6851-6855). Also contemplated, are humanized antibodies, which are generally produced by transferring complementarity determining regions (CDRs) from heavy and light variable chains of a non-human (*e.g.*, rodent, preferably

mouse) immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the non-human counterparts. The use of antibody components derived from humanized antibodies obviates potential problems associated with the immunogenicity of non-human constant regions. General techniques for cloning non-human, particularly murine, immunoglobulin variable domains are described, for example, by Orlandi *et al.* (1989, Proc. Natl. Acad. Sci. USA 86: 3833). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.* (1986, *Nature* 321:522), Carter *et al.* (1992, *Proc. Natl. Acad. Sci. USA* 89: 4285), Sandhu (1992, *Crit. Rev. Biotech.* 12: 437), Singer *et al.* (1993, *J. Immun.* 150: 2844), Sudhir (ed., Antibody Engineering Protocols, Humana Press, Inc. 1995), Kelley ("Engineering Therapeutic Antibodies," in Protein Engineering: Principles and Practice Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Pat. No. 5,693,762 (1997). Humanized antibodies include "primatized" antibodies in which the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest. Also contemplated as antigen-binding molecules are humanized antibodies.

[0066] By "*autologous*" is meant something (*e.g.*, cells, tissues *etc.*) derived from the same organism.

[0067] The term "*allogeneic*" as used herein refers to cells, tissues, organisms *etc.* that are of different genetic constitution.

[0068] By "*biologically active fragment*" is meant a fragment of a full-length parent polypeptide which fragment retains an activity of the parent polypeptide. As used herein, the term "*biologically active fragment*" includes deletion mutants and small peptides, for example of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous amino acids, which comprise an activity of the parent polypeptide. Peptides of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesized using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "*Peptide Synthesis*" by Atherton and Shephard which is included in a publication entitled "*Synthetic Vaccines*" edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

[0069] As used herein, a "*cellular preparation*," refers to a composition comprising at least one cell population as an active ingredient.

[0070] By "*coding sequence*" is meant any nucleic acid sequence that contributes to the code for the polypeptide product of a gene or for the final mRNA product of a gene (*e.g.* the mRNA product of a gene following splicing). By contrast, the term "non-coding sequence" refers to any nucleic acid sequence that does not contribute to the code for the polypeptide product of a gene or for the final mRNA product of a gene.

[0071] Throughout this specification, unless the context requires otherwise, the words "*comprise*," "*comprises*" and "*comprising*" will be understood to imply the inclusion of a stated step

or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0072] The terms "*construct*" and "*synthetic construct*" are used interchangeably herein to refer to heterologous nucleic acid sequences that are operably linked to each other and may include sequences providing the expression of a polynucleotide in a host cell and optionally sequences that provide for the maintenance of the construct.

[0073] By "*corresponds to*" or "*corresponding to*" is meant an antigen which encodes an amino acid sequence that displays substantial sequence identity or similarity to an amino acid sequence in a target antigen. In general, the antigen will display at least about 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 % identity or similarity to at least a portion of the target antigen.

[0074] As used herein, "*culturing*," "*culture*" and the like refer to the set of procedures used *in vitro* where a population of cells (or a single cell) is incubated under conditions which have been shown to support the growth or maintenance of the cells *in vitro*. The art recognizes a wide number of formats, media, temperature ranges, gas concentrations *etc*. which need to be defined in a culture system. The parameters will vary based on the format selected and the specific needs of the individual who practices the methods herein disclosed. However, it is recognized that the determination of culture parameters is routine in nature.

[0075] By "*effective amount*", in the context of stimulating an immune response or treating or preventing a disease or condition, is meant the administration of that amount of composition to an individual in need thereof, either in a single dose or as part of a series, that is effective for that modulation, treatment or prevention. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[0076] By "*expression vector*" is meant any autonomous genetic element capable of directing the synthesis of a protein encoded by the vector. Such expression vectors are known by practitioners in the art.

[0077] The term "*gene*" is used in its broadest context to include both a genomic DNA region corresponding to the gene as well as a cDNA sequence corresponding to exons or a recombinant molecule engineered to encode a functional form of a product.

[0078] The term "*IL-25 receptor*" as used herein means a receptor or a receptor complex mediating IL-25 signaling. IL-25 signaling requires two receptors, IL17RB and IL17RA, which may form a heteromeric complex. IL-25 binds to IL17RB with high affinity, whereas IL17RA

does not bind IL-25 but is required for activating signaling pathways upon ligand binding (*see*, Rickel *et al.*, *J Immunol*, 2008, 181: 4299-310). Thus, "IL-25 receptor" contemplates both IL17RB and IL17RA. The term "IL17RB" (also known as IL-17BR, CRL4, EVI27, IL17RH1, and MGC5245) as used herein means "interleukin 17 receptor B," a polypeptide having an amino acid sequence according to UniProtKB accession no. Q9NRM6, the product of an *IL17RB* gene (*e.g.*, a human *IL17RB* gene (identified by GenBank accession no. AF212365)), and includes all of the variants, isoforms and species homologs of IL17RB. Both IL-25 and IL-17B are ligands for IL17RB, but the receptor binds IL-25 with higher affinity (*see*, Lee, *et al.*, *J Biol Chem*, 2001, 276, 1660-64, 2001). The term "IL17RA," (also known as CD217, IL17R, CDw217, IL-17RA, hIL-17R, and MGC10262) as used herein means "interleukin 17 receptor A," a polypeptide having an amino acid sequence according to UniProt accession no. Q96F46, the product of an *IL17RA* gene (*e.g.*, a human *IL17RA* gene (identified by GenBank accession no. BC011624)), and includes all of the variants, isoforms and species homologs of IL17RA. Variants of IL17RB and IL17RA also include soluble mature receptors.

[0079] The terms "*IL-25*" and "*IL-25 polypeptide*" (also commonly known as IL17E, and IL-17E) as used herein means "*interleukin-25*," a polypeptide having a sequence according to UniProt accession no. Q9H293, the product of an *IL-25* gene (*e.g.*, the human *IL17E* gene (identified by GenBank accession no. AF305200), and includes all of the variants, isoforms and species homologs of IL-25.

[0080] "*IL-25 signaling*" as used herein means the processes initiated by IL-25 or another IL-25 receptor ligand interacting with the IL-25 receptor on the cell surface, resulting in measurable changes in cell function. The IL-25 receptor complex includes IL17RB and IL17RA, and ligand binding activates downstream signal transduction pathways for example adaptor molecule TRAF6, JNK/p38 and ERK, and NFκB, leading to the production of cytokines and chemokines (*see*, Maezawa *et al.*, *J Immunol*, 2006, 176: 1013-18). IL-25 signaling can be measured, for example, by assessing the amount of cytokines and chemokines produced upon induction with an IL-25 receptor ligand, for example measuring production of CXCL-8, IL-6, G-CSF, MCP-1, MIP-1.alpha., RANTES, or CCL2 (as described in Cai *et al.*, *Cytokine*, 2001, 16: 10-21; Lee *et al.*, *J Biol Chem*, 276: 1660-64; Pan *et al.*, *J Immunol*, 2001, 167: 6559-67; Wong *et al.*, *Am J Respir Cell Mol Biol*, 2005, 33: 186-194). The methods and suitable readout systems are well known in the art and are commercially available.

[0081] To enhance immune response ("*immunoenhancement*"), as is well-known in the art, means to increase the animal's capacity to respond to foreign or disease-specific antigens (*e.g.*, cancer antigens) *i.e.*, those cells primed to attack such antigens are increased in number, activity, and ability to detect and destroy those antigens. Strength of immune response is measured by standard tests including: direct measurement of peripheral blood lymphocytes by means known to the art; natural killer cell cytotoxicity assays (see, *e.g.*, Provinciali M. *et al.* (1992, *J. Immunol. Meth.* 155: 19-24), cell proliferation assays (see, *e.g.*, Vollenweider, I. And Groseurth, P. J. (1992, *J. Immunol. Meth.* 149: 133-135), immunoassays of immune cells and subsets (see, *e.g.*, Loeffler, D. A., *et al.* (1992, *Cytom.* 13: 169-174); Rivoltini, L., *et al.* (1992, *Can. Immunol. Immunother.* 34: 241-251); or skin tests for cell-mediated immunity (see, *e.g.*, Chang, A. E. *et al.* (1993, *Cancer Res.* 53: 1043-1050). Any statistically significant increase in strength of immune response as measured by the foregoing tests is considered "*enhanced immune response*",

"*immunoenhancement*" or "*immunopotentiation*" as used herein. Enhanced immune response is also indicated by physical manifestations such as fever and inflammation, as well as healing of systemic and local infections, and reduction of symptoms in disease, *i.e.*, decrease in tumor size, alleviation of symptoms of a disease or condition including, but not restricted to, leprosy, tuberculosis, malaria, aphthous ulcers, herpetic and papillomatous warts, gingivitis, arthrosclerosis, the concomitants of AIDS such as Kaposi's sarcoma, bronchial infections, and the like. Such physical manifestations also define "*enhanced immune response*" "*immunoenhancement*" or "*immunopotentiation*" as used herein.

[0082] Reference herein to "*immuno-interactive*" includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0083] "*Inactivation*" of a cell is used herein to indicate that the cell has been rendered incapable of cell division to form progeny. The cell may nonetheless be capable of response to stimulus, or biosynthesis and/or secretion of cell products such as cytokines. Methods of inactivation are known in the art. Preferred methods of inactivation are treatment with toxins such as mitomycin C, or irradiation. Cells that have been fixed or permeabilized and are incapable of division are also examples of inactivated cells.

[0084] By "*isolated*" is meant material that is substantially or essentially free from components that normally accompany it in its native state.

[0085] A composition is "*immunostimulatory*" if it is capable of either: a) generating an immune response against an antigen (*e.g.*, a tumor antigen) in a naïve individual; or b) reconstituting, boosting, or maintaining an immune response in an individual beyond what would occur if the compound or composition was not administered. A composition is immunogenic if it is capable of attaining either of these criteria when administered in single or multiple doses.

[0086] By "*stimulating*" is meant directly or indirectly increasing the level and/or functional activity of a target molecule. For example, an agent may indirectly stimulate the said level/activity by interacting with a molecule other than the target molecule. In this regard, indirect stimulation of a gene encoding a target polypeptide includes within its scope stimulation of the expression of a first nucleic acid molecule, wherein an expression product of the first nucleic acid molecule stimulates the expression of a nucleic acid molecule encoding the target polypeptide. In certain embodiments, "*stimulation*" or "*stimulating*" means that a desired/selected response is more efficient (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60% or more), more rapid (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60% or more), greater in magnitude (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60% or more), and/or more easily induced (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60% or more) than if the antigen had been used alone.

[0087] The term "*5′ non-coding region*" is used herein in its broadest context to include all nucleotide sequences which are derived from the upstream region of an expressible gene, other than those sequences which encode amino acid residues which comprise the polypeptide product of said gene, wherein 5′ non-coding region confers or activates or otherwise facilitates, at least in part, expression of the gene.

[0088] By "*obtained from*" is meant that a sample such as, for example, a nucleic acid extract or polypeptide extract is isolated from, or derived from, a particular source of the host. For

example, the extract may be obtained from a tissue or a biological fluid isolated directly from the host.

[0089] The term "*oligonucleotide*" as used herein refers to a polymer composed of a multiplicity of nucleotide units (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof) linked *via* phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term "*oligonucleotide*" typically refers to a nucleotide polymer in which the nucleotides and linkages between them are naturally occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule may vary depending on the particular application. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but the term can refer to molecules of any length, although the term "*polynucleotide*" or "*nucleic acid*" is typically used for large oligonucleotides.

[0090] The term "*operably connected*" or "*operably linked*" as used herein means placing a structural gene under the regulatory control of a regulatory polynucleotide such as a promoter, which controls the transcription and optionally translation of the gene. For example, in the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting; *i.e.*, the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting; *i.e.*, the genes from which it is derived.

[0091] The terms "*patient*," "*subject*," "*host*" or "*individual*" used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates, rodents (*e.g.*, mice rats, guinea pigs), lagomorphs (*e.g.*, rabbits, hares), bovines (*e.g.*, cattle), ovines (*e.g.*, sheep), caprines (*e.g.*, goats), porcines (*e.g.*, pigs), equines (*e.g.*, horses), canines (*e.g.*, dogs), felines (*e.g.*, cats), avians (*e.g.*, chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars *etc*), marine mammals (*e.g.*, dolphins, whales), reptiles (*e.g.*, snakes, frogs, lizards *etc*), and fish. A preferred subject is a human in need of treatment or prophylaxis for a condition or disease, which is associated with the presence or aberrant expression of an antigen of interest. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0092] By "*pharmaceutically-acceptable carrier*" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical or systemic administration.

[0093] The term "*pharmaceutically compatible salt*" as used herein refers to a salt which is toxicologically safe for human and animal administration. This salt may be selected from a group including hydrochlorides, hydrobromides, hydroiodides, sulfates, bisulfates, nitrates, citrates,

tartrates, bitartrates, phosphates, malates, maleates, napsylates, fumarates, succinates, acetates, terephthalates, pamoates and pectinates.

[0094] The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotides in length.

[0095] The terms "*polynucleotide variant*" and refers to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The terms "*polynucleotide variant*" and "*variant*" also include naturally occurring allelic variants.

[0096] "*Polypeptide*," "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0097] The term "*peptide variant,*" "*polypeptide variant,*" and "*variant*" refer to polypeptides which vary from a reference polypeptide by the addition, deletion or substitution of at least one amino acid. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Preferred variant polypeptides comprise conservative amino acid substitutions. Exemplary conservative substitutions in a polypeptide may be made according to the following table:

TABLE A

ORIGINAL RESIDUE	*Exemplary Substitutions*
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,

Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

[0098] The term "*inactive variant*" refers to a polypeptide which varies from a reference polypeptide by the addition, deletion or substitution of at least one amino acid that is important in conferring activity to the polypeptide. It is well understood in the art that some amino acids may be changed to disrupt critical interactions and therefore change the nature of the activity of the polypeptide. Suitably, an inactive variant polypeptide will have only around 50%, 45%, 40%, 35%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%%, 1%, less than 1% or 0% activity of the corresponding wild-type polypeptide.

[0099] Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE A. Other replacements would be non-conservative substitutions and relatively fewer of these may be tolerated. Generally, the substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (*e.g.*, Ser or Asn) is substituted for, or by, a hydrophobic residue (*e.g.*, Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (*e.g.*, Arg, His or Lys) is substituted for, or by, an electronegative residue (*e.g.*, Glu or Asp) or (d) a residue having a smaller side chain (*e.g.*, Ala, Ser) or no side chain (*e.g.*, Gly) is substituted for, or by, one having a bulky side chain (*e.g.*, Phe or Trp).

[0100] Reference herein to a "*promoter*" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (*i.e.*, upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or environmental stimuli, or in a tissue-specific or cell-type-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5′, of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. Preferred promoters according to the invention may contain additional copies of one or more specific regulatory elements to further enhance expression in a cell, and/or to alter the timing of expression of a structural gene to which it is operably connected.

[0101] The term "*recombinant polynucleotide*" as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

[0102] By "*recombinant polypeptide*" is meant a polypeptide made using recombinant techniques, *i.e.*, through the expression of a recombinant polynucleotide.

[0103] The term "*sequence identity*" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "*percentage of sequence identity*" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0104] As used herein "*stimulating*" an immune or immunological response refers to administration of a composition that initiates, boosts, or maintains the capacity for the host's immune system to react to a target substance or antigen, such as a foreign molecule, an allogeneic cell, or a tumor cell, at a level higher than would otherwise occur. Stimulating a "*primary*" immune response refers herein to eliciting specific immune reactivity in a subject in which previous reactivity was not detected; for example, due to lack of exposure to the target antigen, refractoriness to the target, or immune suppression. Stimulating a "*secondary*" response refers to the reinitiation, boosting, or maintenance of reactivity in a subject in which previous reactivity was detected; for example, due to natural immunity, spontaneous immunization, or treatment using one or several compositions or procedures.

[0105] As used herein, the term "*suppress*" or "*suppressing*" means partially or totally blocking stimulation, decreasing, preventing, delaying activation, inactivating, desensitizing, inhibiting, or down regulating a measureable change in IL-25 signaling. For example, suppressing IL-25 signaling can be achieved by blocking IL-25 and IL-25 receptor interaction or suppressing IL-25 receptor expression.

[0106] The term "*Th17*" refers to a subclass of T helper cells that produce *inter alia* IL-17A, IL-17F, IL-21, IL-22, IL-26, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), GM-CSF, and MIP-A, and which elicit inflammatory reactions associated with a cellular, *i.e.*, non-immunoglobulin, response to a challenge. Thus, a Th17 cytokine response encompasses an immune response whose most prominent feature comprises abundant $CD4^+$ helper T cell activation that is associated with increased levels of Th17 cytokines (*e.g.*, IL-17A, IL-17F, IL-21, IL-22, IL-26, IFN-γ, TNF-α, *etc.*) relative to these cytokine amounts in the absence of activation. A Th17 cytokine response can also refer to the production of cytokines from other white blood cells and non-white blood cells. A Th17 cytokine response can include abundant $CD8^+$ cytotoxic T lymphocyte activity including cytokine production, and the activation of type 1 T cytotoxic cells (including ILC1). A Th17 response is typically promoted by $CD4^+$ "*Th17*" T-helper cells however a Th17 response can include $CD8^+$ type 1 T cytotoxic cells.

[0107] By "*treatment*, " "*treat*," "*treated*" and the like is meant to include both prophylactic and therapeutic treatment, including but not limited to preventing, relieving, altering, reversing, affecting, inhibiting the development or progression of, ameliorating, or curing (1) a disease or condition associated with the presence or aberrant expression of a target antigen, or (2) a symptom of the disease or condition, or (3) a predisposition toward the disease or condition, including conferring protective immunity to a subject.

[0108] By "*vector*" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are well known to those of skill in the art.

2. Compositions

[0109] The present invention is predicated at least in part on the determination that expression of the cytokine IL-25 plays an important role in regulating the functional avidity of innate lymphoid cells (ILC), and that T cell avidity is improved by inhibition of IL-25 function in the local milieu of the immune response, leading the inventors to discover that a subject's T cell mediated immune response may be enhanced by removal, inhibition or neutralization of IL-25 production and/or function in the local milieu of the immune response.

[0110] Based on these observations, the present inventors propose that more efficacious prophylactic or therapeutic immune responses to a target antigen can be achieved using compositions that comprise a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject, together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible. In specific embodiments, the compositions are introduced into antigen-presenting cells such that the first and second agents are co-located in or on or co-presented by the antigen-presenting cells. In some specific embodiments, the first agent and the second agent are not fused or conjugated to one another, but present as separate components in the composition.

2.1 Inhibitors of IL-25 function

[0111] The inhibitor of IL-25 function includes any molecule or compound that directly or indirectly binds or physically associates with IL-25 or its receptor(s) (*e.g.*, IL-25R) and that suitably blocks, inhibits or otherwise antagonizes at least one of its functions or activities (*e.g.*, binding to or interaction with one or more surface molecules (*e.g.*, receptors) present on white blood cells, especially lymphocytes and more especially ILC2). The binding or association may involve the formation of an induced magnetic field or paramagnetic field, covalent bond formation,

an ionic interaction such as, for example, occur in an ionic lattice, a hydrogen bond or alternatively, a van der Waals interaction such as, for example, a dipole-dipole interaction, dipole-induced-dipole interaction, induced-dipole-induced-dipole interaction or a repulsive interaction or any combination of the above forces of attraction.

[0112] In certain embodiments, the inhibitor of IL-25 function is any molecule capable of specifically preventing activation of cellular receptors for IL-25. For example, inhibitors of this type can be selected from soluble, membrane-bound or defective IL-25 receptors or soluble IL-25 receptor subunits, including but not limited to IL-17 receptor A (IL17RA) and IL-17 receptor B (IL17RB) polypeptides. An illustrative IL17RA polypeptide sequence is shown in SEQ ID NO: 1 of U.S. Patent No. 6,072,033 and deposited as UniProtKB accession no. Q96F46:

MGAARSPPSAVPGPLLGLLLLLLGVLAPGGASLRLLDHRALVCSQPGLNCTVKNSTCLDDSWIHPRNLTP
SSPKDLQIQLHFAHTQQGDLFPVAHIEWTLQTDASILYLEGAELSVLQLNTNERLCVRFEFLSKLRHHHRR
WRFTFSHFVVDPDQEYEVTVHHLPKPIPDGDPNHQSKNFLVPDCEHARMKVTTPCMSSGSLWDPNITVE
TLEAHQLRVSFTLWNESTHYQILLTSFPHMENHSCFEHMHHIPAPRPEEFHQRSNVTLTLRNLKGCCRHQ
VQIQPFFSSCLNDCLRHSATVSCPEMPDTPEPIPDYMPLWVYWFITGISILLVGSVILLIVCMTWRLAGPG
SEKYSDDTKYTDGLPAADLIPPPLKPRKVWIIYSADHPLYVDVVLKFAQFLLTACGTEVALDLLEEQAISEA
GVMTWVGRQKQEMVESNSKIIVLCSRGTRAKWQALLGRGAPVRLRCDHGKPVGDLFTAAMNMILPDFK
RPACFGTYVVCYFSEVSCDGDVPDLFGAAPRYPLMDRFEEVYFRIQDLEMFQPGRMHRVGELSGDNYLRS
PGGRQLRAALDRFRDWQVRCPDWFECENLYSADDQDAPSLDEEVFEEPLLPPGTGIVKRAPLVREPGSQ
ACLAIDPLVGEEGGAAVAKLEPHLQPRGQPAPQPLHTLVLAAEEGALVAAVEPGPLADGAAVRLALAGEGE
ACPLLGSPGAGRNSVLFLPVDPEDSPLGSSTPMASPDLLPEDVREHLEGLMLSLFEQSLSCQAQGGCSRP
AMVLTDPHTPYEEEQRQSVQSDQGYISRSSPQPPEGLTEMEEEEEEEQDPGKPALPLSPEDLESLRSLQR
QLLFRQLQKNSGWDTMGSESEGPSA [SEQ ID NO:1]

[0113] IL-17B and its many isoforms are known in the art, such as those disclosed and described in Tial *et* al., Oncogene, 19:2098 (2000). By way of an illustrative example, a suitable inhibitor of IL-25 function of this type is a soluble fragment of a human IL17RB polypeptide (*e.g.*, the human IL17RB polypeptide deposited as UniProt accession no. Q9NRM6 and as set forth in SEQ ID NO: 2, below).

MSLVLLSLAALCRSAVPREPTVQCGSETGPSPEWMLQHDLIPGDLRDLRVEPVTTSVATGDYSILMNVSW
VLRADASIRLLKATKICVTGKSNFQSYSCVRCNYTEAFQTQTRPSGGKWTFSYIGFPVELNTVYFIGAHNI
PNANMNEDGPSMSVNFTSPGCLDHIMKYKKKCVKAGSLWDPNITACKKNEETVEVNFTTTPLGNRYMAL
IQHSTIIGFSQVFEPHQKKQTRASVVIPVTGDSEGATVQLTPYFPTCGSDCIRHKGTVVLCPQTGVPFPLD
NNKSKPGGWLPLLLLSLLVATWVLVAGIYLMWRHERIKKTSFSTTTLLPPIKVLVVYPSEICFHHTICYFTEF
LQNHCRSEVILEKWQKKKIAEMGPVQWLATQKKAADKVVFLLSNDVNSVCDGTCGKSEGSPSENSQDL
FPLAFNLFCSDLRSQIHLHKYVVVYFREIDTKDDYNALSVCPKYHLMKDATAFCAELLHVKQQVSAGKRS
QACHDGCCSL [SEQ ID NO:2]

[0114] More specifically, a particularly suitable soluble IL17RB polypeptide may lack the native transmembrane domain (*i.e.*, lacking amino acid residues 293-313 of the wild-type human IL17RB polypeptide set forth in SEQ ID NO: 2). Accordingly, in some embodiments, the inhibitor of IL-25 function is an IL17RB polypeptide that comprises the extracellular domain (corresponding to amino acid residues 18-292 of the sequence set forth in SEQ ID NO: 2) or a fragment thereof.

Optionally, the IL17RB polypeptide may also comprises the native signal sequence (*i.e.*, residues 1-17 of the amino acid sequence set forth in SEQ ID NO: 2).

[0115] Thus, certain aspects of the present invention are drawn to agents (*e.g.*, antigen-binding proteins) that block the association of IL-17RA with IL-17RB (and/or with additional receptor subunits) and thereby preventing formation of a functional receptor complex (*i.e.*, a receptor complex that is capable of being activated). Illustrative examples of suitable inhibitors of this type are disclosed in U.S. Patent Publication No. 2014/0322238, all of which are incorporated herein by reference. For example, illustrative examples of suitable antibodies that bind IL-17RA are listed in TABLE B:

TABLE B

Antibody	SEQ ID NO:	Description	SEQ ID NO:	Description
3.1404.1	3	AM_H14 Vh	9	AM_L14 Vl
4.361.1	4	AM_H17 Vh	10	AM_L17 Vl
4.16.1	5	AM_L19 Vh	11	AM_L19 Vl
381.1.1	6	AM_L22 Vh	12	AM_L22 Vl
3.454.1	7	AM_H23 Vh	13	AM_L23 Vl
3.454.1.1	8	AM_H24 Vh	14	AM_L24 Vl

wherein:

SEQ ID NO: 3 is:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYGISWVRQAPGQGLEWMGWISTYSGNTNYAQKLQGR
VTMTTDTSTSTAYMELRSLRSDDTAVYYCARRQLYFDYWGQGTLVTVSS;

SEQ ID NO: 4 is:
QVQLVQSGAEVKKPGAAVKVSCKATGYTLTSYGISWVRQAPGQGLEWMGWISAYSGNTKYAQKLQGR
VTMTTDTSTSTAYMELRSLRSDDTAVYYCARKQLVFDYWGQGTLVTVSS;

SEQ ID NO: 5 is:
QVQLVQSGAEVKKPGASVKVSCKASGYTLTSYGISWVRQAPGQGLEWMGWISAYSGNTKYAQKFQGR
VTMTTDTSTSTAYMELRSLRSDDTAVYYCARRQLALDYWGQGTLVTVSS;

SEQ ID NO: 6 is:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYGISWVRQAPGQGLEWMGWISAYSGNTNYAQKLQGR
VTMTTDTSTSTAYMELRSLRSDDTAVYYCARRQLYFDYWGQGTLVTVSS;

SEQ ID NO: 7 is:
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPAGKRLEWIGRIYPSGRTNYNPSLKSRVTMS
VDTSKNQFSLKLSSVTAADTAVYYCAREAYELQLGLYYYYGMDVWGQGTPVTVSS;

SEQ ID NO: 8 is:
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQAAGKRLEWIGRIYPSGRTNYNPSLKSRVTM
SVDTSKNQFSLKLSSVTAADTAVYYCAREAYELQLGLYYYYGMDVWGQGTPVTVSS;

SEQ ID NO: 9 is:
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWFQQKPGQAPRPLIYDASTRATGVPARFSGSGSGT
DFTLTISSLQSEDFAVYYCQQYDNWPLTFGGGTKVEIK;

SEQ ID NO: 10 is:
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTE
FTLTISSLQSEDFAVYSCQQYDNWPLTFGGGTKVEIK;

SEQ ID NO: 11 is:
EIVMTQSPATLSVSPGERATLSCRASQSISSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSDNGSGTE
FTLTISSLQSEDFAVYFCQQYDTWPLTFGGGTKVEIK;

SEQ ID NO: 12 is:
EIVMTQSPATLSVSPGERVTLSCRASQSVSSNLAWFQQKPGQAPRPLIYDASTRAAGIPARFSGSGSGTD
FTLTISSLQSEDFAVYYCQQYDNWPLTFGGGTKVEIK;

SEQ ID NO: 13 is:
DIQMTQSPSSLSASVGDRVTISCRASQGIINDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGT
EFTFTISSLQPEDFATYYCLQHNSYPPTFGQGTKVEIK; and

SEQ ID NO: 14
DIVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGHTCLNWFQQRPGQSPRRLIYKVSNWDSGVPDRFSG
SGSGTDFTLKISRVEADDVGVYYCMQGTHWPLCSFGQGTKLEI.

[0116] In certain embodiments, the inhibitor of IL-25 function is an inactive variant form of IL-25. For example, an inactive variant IL-25 polypeptides may be distinguished from a wild-type IL-25 polypeptide by one or more amino acids. By way of an illustrative example, a suitable wild-type polypeptide is the human IL-25 polypeptides, as identified by UniProt accession no. Q9H293, and as set forth below:

MRERPRLGEDSSLISLFLQVVAFLAMVMGTHTYSHWPSCCPSKGQDTSEELLRWSTVPVPPLEPARPNRH
PESCRASEDGPLNSRAISPWRYELDRDLNRLPQDLYHARCLCPHCVSLQTGSHMDPRGNSELLYHNQTV
FYRRPCHGEKGTHKGYCLERRLYRVSLACVCVRPRVMG [SEQ ID NO: 15].

[0117] Alternatively, such an inhibitor can be an antigen-binding molecule that is immuno-interactive with IL-25 receptor. In these embodiments, the antigen-binding molecule may bind to the IL-25 receptor but will not signal via the receptor, thus blocking any host IL-25 signaling (such antigen-binding molecules are also referred to herein as antagonistic antigen-binding moelcules). In other embodiments, the inhibitor of IL-25 function is an antigen-binding molecule that is immuno-interactive with at least a portion of IL-25. In these embodiments, the antigen-binding molecules can be immuno-interactive with an active or an inactive form of IL-25, the difference being that antigen-binding molecules to the active cytokine are more likely to recognize epitopes that are only present in the active conformation. Representative examples of such inhibitors include the antibodies disclosed in U.S. Patent Nos. 8,785,605 and 8,658,169, and U.S. Pat. Appl. Pub. No. 2016/0083466.

[0118] By way of a specific example, one such IL-25 antibody comprises:

(a) an antibody VL domain comprising a CDR1 having the amino acid sequence SASQGISNYLN [SEQ IDNO:102], and CDR2 having the amino acid sequence YTSSLHS [SEQ IDNO:103], and a CDR3 having the amino acid sequence QQYLAFPYTF [SEQ IDNO:104]; and

(b) an antibody VH domain comprising a CDR1 having the amino acid sequence GYTMN [SEQ IDNO:105], a CDR2 having the amino acid sequence LINPYNGGTSYNQNFKG [SEQ IDNO:106], and a CDR3 having the amino acid sequence EDYDGYLYFAMY [SEQ IDNO:107].

[0119] By way of a specific example, one such IL-25 antibody comprises a VL domain comprising the amino acid sequence set forth in SEQ ID NO:16 and a VH domain comprising SEQ ID NO: 17 (*i.e.*, the "M6" antibody),

wherein:

SEQ ID NO: 16 is:
DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKADGTVELLIYYTSSLHSGVPSRFSGSGSGT
DYSLTISNLEPEDIATYYCQQYSKLPYTFGGGTKLEIK; and

SEQ ID NO: 17 is:
EVQLQQSGPELVKPGASMKISCKASGYSFTDYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQNFKGKA
TLTVDKSSSTAYMELLSLTSEDSAVYYCAREGYDGYLYFAMDYWGQGTSVTVS.

[0120] In alternative embodiments, the IL-25 antibody binds one or more amino acid sequences of human IL-25 selected from the group SEDGPLNSRAISPWRY, DLNRLPQDLYHARCLCPHC, and RLYRVSL.

[0121] Yet other examples of suitable antibodies comprise:

(a) an antibody VL domain comprising a CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33; a CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 34, 35, 36, 37, 38 and 39; and a CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53 and 54; and

(b) an antibody VH domain comprising a CDR1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 and 70; a CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81 and 82; a CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 97, 98 and 99,

wherein:

SEQ ID NO: 18 is: QSIGSY;	SEQ ID NO: 23 is: QSISSY;	SEQ ID NO: 28 is: QSVLDSSNNKN;
SEQ ID NO: 19 is: QSISSY;	SEQ ID NO: 24 is: QGITNY;	SEQ ID NO: 29 is: QSVLDSSNNKNY;
SEQ ID NO: 20 is: QSVFLGSNNKNY;	SEQ ID NO: 25 is: QNINSH;	SEQ ID NO: 30 is: QNVLITSNNKNY;
SEQ ID NO: 21 is: QSVLYSSNNKNY;	SEQ ID NO: 26 is: QNILLTSSNKNY;	SEQ ID NO: 31 is: QTIYSY;
SEQ ID NO: 22 is: QDINNY;	SEQ ID NO: 27 is: QDINSY;	SEQ ID NO: 32 is: QSILYNSDNKNY;

SEQ ID NO: 33 is: QDISSF;

SEQ ID NO: 34 is: AAS;

SEQ ID NO: 35 is: WAS;

SEQ ID NO: 36 is: WSS;

SEQ ID NO: 37 is: STS;

SEQ ID NO: 38 is: TAS;

SEQ ID NO: 39 is: DAS;

SEQ ID NO: 40 is: QQTYSTPIT;

SEQ ID NO: 41 is: QQYFITPLT;

SEQ ID NO: 42 is: QQYFSTPWT;

SEQ ID NO: 43 is: QQTFITPLT;

SEQ ID NO: 44 is: QQSYLTPLT;

SEQ ID NO: 45 is: QQYGSAPWT;

SEQ ID NO: 46 is: QQTYITPLT;

SEQ ID NO: 47 is: QQYYITPFT;

SEQ ID NO: 48 is: QHLSSFPPT;

SEQ ID NO: 49 is: QQYYFTPLT;

SEQ ID NO: 50 is: QQFYNSPWT;

SEQ ID NO: 51 is: QQYYSTPFT;

SEQ ID NO: 52 is: QQTYSTPLT;

SEQ ID NO: 53 is: QQYFFTPFT;

SEQ ID NO: 36 is: WSS;

SEQ ID NO: 37 is: STS;

SEQ ID NO: 38 is: TAS;

SEQ ID NO: 39 is: DAS;

SEQ ID NO: 40 is: QQTYSTPIT;

SEQ ID NO: 41 is: QQYFITPLT;

SEQ ID NO: 54 is: QQVNSYPIT;

SEQ ID NO: 55 is: GFTFSNYD;

SEQ ID NO: 56 is: GFTFSNAW;

SEQ ID NO: 57 is: GYTFTSYG;

SEQ ID NO: 58 is: GYTFTNYG;

SEQ ID NO: 59 is: GYTFSSYG;

SEQ ID NO: 60 is: GGSISSHY;

SEQ ID NO: 61 is: GGSISSYF;

SEQ ID NO: 62 is: GGSISNYF;

SEQ ID NO: 63 is: GGSISSYY;

SEQ ID NO: 64 is: GGSISDYY;

SEQ ID NO: 65 is: GGSINSYY;

SEQ ID NO: 66 is: GGSINSYS;

SEQ ID NO: 67 is: GASISNYY;

SEQ ID NO: 68 is: DFAFTTYG;

SEQ ID NO: 69 is: EYTFSNYG;

SEQ ID NO: 70 is: SGSIRSSNYY;

SEQ ID NO: 71 is: IERKTDGGTT;

SEQ ID NO: 72 is: ARVPITGTTWFDP;

SEQ ID NO: 73 is: IYYSGST;

SEQ ID NO: 74 is: IFYSGNT;

SEQ ID NO: 75 is: IDYSGST;

SEQ ID NO: 76 is: ISAYNDNT;

SEQ ID NO: 77 is: NYNSGST;

SEQ ID NO: 78 is: ISAYNGNT;

SEQ ID NO: 79 is: IGAYSGFT;

SEQ ID NO: 80 is: IYYSGNT;

SEQ ID NO: 81 is: IYNSENT;

SEQ ID NO: 82 is: IGSAGDT;

SEQ ID NO: 83 is: TTVGPYSVPFDY;

SEQ ID NO: 84 is: ARVPITGTTWFDP;

SEQ ID NO: 85 is: ARVRFSDYELNWFDP;

SEQ ID NO: 86 is: ARVPLQWFGESF;

SEQ ID NO: 87 is: ARVGTGTDSYFDF;

SEQ ID NO: 92 is: ARDPDYCSSNTCSDAFDL;

SEQ ID NO: 96 is: ARQGYSDYELNWFDP;

SEQ ID NO: 88 is: ARVPITGTTSSFDF;

SEQ ID NO: 89 is: ARHDYNDYELNYFDL;

SEQ ID NO: 90 is: ARQEIINFELNWFDP;

SEQ ID NO: 91 is: ARGYNWNYEIAWFDP;

SEQ ID NO: 93 is: ARHYFDSGTYELGY;

SEQ ID NO: 94 is: ARDGYSSSSGFYYFGM;

SEQ ID NO: 95 is: ARGVIWNYELREF;

SEQ ID NO: 97 is: ARTYNWNYEIGAM;

SEQ ID NO: 98 is: ARHDSDYELYGMDV; and

SEQ ID NO: 99 is: ARGDNWNYVSWFF.

[0122] By way of yet another specific example, another suitable antibody is the "2C3" antibody as disclosed in the International Patent Publication No WO2008/129263, in which the IL-25 antibody comprises a VL domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and/or a VH domain comprising the amino acid sequence set forth in SEQ ID NO: 17. In some embodiments, the inhibitor of IL-25 function is an antisense or siRNA molecule designed against IL-25 gene or an IL-25 receptor gene (for example, such as IL-25 siRNA (catalogue sc-105567) available from Santa Cruz Biotechnology, Texas, U.S.A.).

[0123] Peptides, oligonucleotides, or small molecules blocking the interaction between IL-25 and the IL-25 receptor can be used. Such agents and inhibitors can also be peptides, proteins, fusion proteins, or small molecules that prevent interaction of IL-25 with an IL-25 receptor. Agents inhibiting IL-25 function that are suitable for use with the present invention have been previously described, for example, the peptide IL-25 variants disclosed in U.S. Patent No. 6,562,578, and the IL17R like proteins disclosed in U.S. Patent No. 6,635,443.

2.2 Immune-stimulating agents

2.2.1 Antigens

[0124] The present invention contemplates the use of the compositions of the invention of an immune stimulator comprising any antigen that corresponds to at least a portion of a target antigen of interest for stimulating an immune response to the target antigen. The antigen that corresponds to at least a portion of the target antigen may be in soluble form (*e.g.*, a peptide or polypeptide) when expressed.

[0125] Target antigens useful in the present invention are typically proteinaceous molecules, representative examples of which include polypeptides and peptides. Target antigens may be selected from endogenous antigens produced by a host or exogenous antigens that are foreign to the host. Suitable endogenous antigens include, but are not restricted to, cancer or tumor antigens. Non-limiting examples of cancer or tumor antigens include antigens from a cancer or tumor selected from ABL1 proto-oncogene, AIDS related cancers (*e.g.*, Kaposi sarcoma), acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell

lymphoma, dermatofibrosarcoma protuberans, desmoplastic small round cell tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's Sarcoma, extra-hepatic bile duct cancer, eye cancer, melanoma, retinoblastoma, fallopian tube cancer, Fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynecological cancers, hematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid tumor of kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer (NSCLC), ocular cancers, esophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal tumors, pituitary cancer, polycythemia vera, prostate cancer, rare cancers and associated disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (SCLC), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's macroglobulinemia, Wilms' tumor. In certain embodiments, the cancer or tumor relates to nasopharyngeal cancer. Illustrative examples of nasopharyngeal cancer antigens include EBNA-1, LMP-1, LMP-2, or a combination thereof. Other tumor-specific antigens include, but are not limited to: etv6, aml1, cyclophilin b (acute lymphoblastic leukemia); Ig-idiotype (B cell lymphoma); E-cadherin, α-catenin, β-catenin, γ-catenin, p120ctn (glioma); p21ras (bladder cancer); p21ras (biliary cancer); MUC family, HER2/neu, c-erbB-2 (breast cancer); p53, p21ras (cervical carcinoma); p21ras, HER2/neu, c-erbB-2, MUC family, Cripto-1protein, Pim-1 protein (colon carcinoma); Colorectal associated antigen (CRC)-CO17-1A/GA733, APC (colorectal cancer); carcinoembryonic antigen (CEA) (colorectal cancer; choriocarcinoma); cyclophilin b (epithelial cell cancer); HER2/neu, c-erbB-2, ga733 glycoprotein (gastric cancer); α-fetoprotein (hepatocellular cancer); Imp-1, EBNA-1 (Hodgkin's lymphoma); CEA, MAGE-3, NY-ESO-1 (lung cancer); cyclophilin b (lymphoid cell-derived leukemia); melanocyte differentiation antigen (*e.g.*, gp100, MART, Melan-A/MART-1, TRP-1, Tyros, TRP2, MC1R, MUC1F, MUC1R or a combination thereof) and melanoma-specific antigens (*e.g.*, BAGE, GAGE-1, gp100In4, MAGE-1 (*e.g.*, GenBank Accession No. X54156 and AA494311), MAGE-3, MAGE4, PRAME, TRP2IN2, NYNSO1a, NYNSO1b, LAGE1, p97 melanoma antigen (*e.g.*, GenBank Accession No. M12154) p5 protein, gp75, oncofetal antigen, GM2 and GD2 gangliosides, cdc27, p21ras, $gp100^{Pmel117}$ or a combination thereof (melanoma); MUC family, p21ras (myeloma); HER2/neu, c-erbB-2 (non-small cell lung carcinoma); MUC family, HER2/neu,

c-erbB-2, MAGE-A4, NY-ESO-1 (ovarian cancer); Prostate Specific Antigen (PSA) and its antigenic epitopes PSA-1, PSA-2, and PSA-3, PSMA, HER2/neu, c-erbB-2, ga733 glycoprotein (prostate cancer); HER2/neu, c-erbB-2 (renal cancer); viral products such as human papillomavirus proteins (squamous cell cancers of the cervix and esophagus); NY-ESO-1 (testicular cancer); and HTLV-1 epitopes (T cell leukemia).

[0126] Foreign or exogenous antigens are suitably selected from antigens of pathogenic organisms. Exemplary pathogenic organisms include, but are not limited to, viruses, bacteria, fungi, parasites, algae and protozoa and amoebae. Illustrative viruses include viruses responsible for diseases including, but not limited to, measles, mumps, rubella, poliomyelitis, hepatitis A, B (*e.g.*, GenBank Accession No. E02707), and C (*e.g.*, GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (*e.g.*, types 4 and 7), rabies (*e.g.*, GenBank Accession No. M34678), yellow fever, Epstein-Barr virus and other herpesviruses such as papillomavirus, Ebola virus, influenza virus, Japanese encephalitis (*e.g.*, GenBank Accession No. E07883), dengue (*e.g.*, GenBank Accession No. M24444), hantavirus, Sendai virus, respiratory syncytial virus, orthomyxoviruses, vesicular stomatitis virus, visna virus, cytomegalovirus and human immunodeficiency virus (HIV) (*e.g.*, GenBank Accession No. U18552). Any suitable antigen derived from such viruses are useful in the practice of the present invention. For example, illustrative retroviral antigens derived from HIV include, but are not limited to, antigens such as gene products of the *gag*, *pol*, and *env* genes, the Nef protein, reverse transcriptase, and other HIV components. Illustrative examples of hepatitis viral antigens include, but are not limited to, antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, *e.g.*, hepatitis A, B, and C, viral components such as hepatitis C viral RNA. Illustrative examples of influenza viral antigens include; but are not limited to, antigens such as hemagglutinin and neuraminidase and other influenza viral components. Illustrative examples of measles viral antigens include, but are not limited to, antigens such as the measles virus fusion protein and other measles virus components. Illustrative examples of rubella viral antigens include, but are not limited to, antigens such as proteins E1 and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components. Illustrative examples of Cytomegaloviral antigens include, but are not limited to, antigens such as envelope glycoprotein B and other Cytomegaloviral antigen components. Non-limiting examples of respiratory syncytial viral antigens include antigens such as the RSV fusion protein, the M2 protein and other respiratory syncytial viral antigen components. Illustrative examples of herpes simplex viral antigens include, but are not limited to, antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components. Non-limiting examples of varicella zoster viral antigens include antigens such as 9PI, gpII, and other varicella zoster viral antigen components. Non-limiting examples of Japanese encephalitis viral antigens include antigens such as proteins E, M-E, M-E-NS 1, NS 1, NS 1-NS2A, and other Japanese encephalitis viral antigen components. Representative examples of rabies viral antigens include, but are not limited to, antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components. Illustrative examples of papillomavirus antigens include, but are not limited to, the L1 and L2 capsid proteins as well as the E6/E7 antigens associated with cervical cancers (*see* Fundamental Virology, Second Edition, eds. Fields, B.N. and Knipe, D.M., 1991, Raven Press, New York, for additional examples of viral antigens).

[0127] Illustrative examples of fungi include Acremonium spp., Aspergillus spp., Basidiobolus spp., Bipolaris spp., Blastomyces dermatitidis, Candida spp., Cladophialophora carrionii, Coccidioides immitis, Conidiobolus spp., Cryptococcus spp., Curvularia spp., Epidermophyton spp., Exophiala jeanselmei, Exserohilum spp., Fonsecaea compacta, Fonsecaea pedrosoi, Fusarium oxysporum, Fusarium solani, Geotrichum candidum, Histoplasma capsulatum var. capsulatum, Histoplasma capsulatum var. duboisii, Hortae werneckii, Lacazia loboi, Lasiodiplodia theobromae, Leptosphaeria senegalensis, Madurella grisea, Madurella mycetomatis, Malassezia furfur, Microsporum spp., Neotestudina rosati, Onychocola canadensis, Paracoccidioides brasiliensis, Phialophora verrucosa, Piedraia hortae, Pityriasis versicolor, Pseudallescheria boydii, Pyrenochaeta romeroi, Rhizopus arrhizus, Scopulariopsis brevicaulis, Scytalidium dimidiatum, Sporothrix schenckii, Trichophyton spp., Trichosporon spp., Zygomycota fungi, Absidia corymbifera, Rhizomucor pusillus and Rhizopus arrhizus. Thus, representative fungal antigens that can be used in the compositions and methods of the present invention include, but are not limited to, candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidioses fungal antigens such as spherule antigens and other coccidioses fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidioses fungal antigen components.

[0128] Illustrative examples of bacteria include bacteria that are responsible for diseases including, but not restricted to, diphtheria (*e.g.*, *Corynebacterium diphtheria*), pertussis (*e.g.*, *Bordetella pertussis*, GenBank Accession No. M35274), tetanus (*e.g.*, *Clostridium tetani*, GenBank Accession No. M64353), tuberculosis (*e.g.*, *Mycobacterium tuberculosis*), bacterial pneumonias (*e.g.*, *Haemophilus influenzae*.), cholera (*e.g.*, *Vibrio cholerae*), anthrax (*e.g.*, *Bacillus anthracis*), typhoid, plague, shigellosis (*e.g.*, *Shigella dysenteriae*), botulism (*e.g.*, *Clostridium botulinum*), salmonellosis (*e.g.*, GenBank Accession No. L03833), peptic ulcers (*e.g.*, *Helicobacter pylori*), Legionnaire's Disease, Lyme disease (*e.g.*, GenBank Accession No. U59487). Other pathogenic bacteria include Escherichia coli, Clostridium perfringens, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes. Thus, bacterial antigens which can be used in the compositions and methods of the invention include, but are not limited to: pertussis bacterial antigens such as pertussis toxin, filamentous hemagglutinin, pertactin, FIM2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diphtheria bacterial antigens such as diphtheria toxin or toxoid and other diphtheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components, streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial antigens such as lipopolysaccharides and other gram-negative bacterial antigen components; Mycobacterium tuberculosis bacterial antigens such as mycolic acid, heat shock protein 65 (HSP65), antigen 85A, the 30kDa major secreted protein (also known as, antigen 85B), and other mycobacterial antigen components; Helicobacter pylori bacterial antigen components, pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pneumococcal bacterial antigen components; Haemophilus influenza bacterial antigens such as capsular polysaccharides and other Haemophilus influenza bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; rickettsiae bacterial antigens such as rompA and other rickettsiae bacterial antigen component. Also included with the

bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens.

[0129] Illustrative examples of protozoa include protozoa that are responsible for diseases including, but not limited to, malaria (*e.g.*, GenBank Accession No. X53832), hookworm, onchocerciasis (*e.g.*, GenBank Accession No. M27807), schistosomiasis (*e.g.*, GenBank Accession No. LOS 198), toxoplasmosis, trypanosomiasis, leishmaniasis, giardiasis (GenBank Accession No. M33641), amoebiasis, filariasis (*e.g.*, GenBank Accession No. J03266), borreliosis, and trichinosis. Thus, protozoal antigens which can be used in the compositions and methods of the invention include, but are not limited to: plasmodium falciparum antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasmal antigen components; schistosomae antigens such as glutathione-S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmaniae antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and trypanosoma cruzi antigens such as the 75-77 kDa antigen, the 56 kDa antigen and other trypanosomal antigen components.

[0130] The present invention also contemplates toxin components as antigens. Illustrative examples of toxins include, but are not restricted to, staphylococcal enterotoxins, toxic shock syndrome toxin; retroviral antigens (*e.g.*, antigens derived from HIV), streptococcal antigens, staphylococcal enterotoxin-A (SEA), staphylococcal enterotoxin-B (SEB), staphylococcal enterotoxin$_{I\text{-}3}$ ($SE_{1\text{-}3}$), staphylococcal enterotoxin-D (SED), staphylococcal enterotoxin-E (SEE) as well as toxins derived from mycoplasma, mycobacterium, and herpes viruses.

[0131] Peptide antigens may be of any suitable size that can be utilized to stimulate or inhibit an immune response to a target antigen of interest. A number of factors can influence the choice of peptide size. For example, the size of a peptide can be chosen such that it includes, or corresponds to the size of, T cell epitopes and/or B cell epitopes, and their processing requirements. Practitioners in the art will recognize that class I-restricted T cell epitopes are typically between 8 and 10 amino acid residues in length and if placed next to unnatural flanking residues, such epitopes can generally require 2 to 3 natural flanking amino acid residues to ensure that they are efficiently processed and presented. Class II-restricted T cell epitopes usually range between 12 and 25 amino acid residues in length and may not require natural flanking residues for efficient proteolytic processing although it is believed that natural flanking residues may play a role. Another important feature of class II-restricted epitopes is that they generally contain a core of 9-10 amino acid residues in the middle which bind specifically to class II MHC molecules with flanking sequences either side of this core stabilizing binding by associating with conserved structures on either side of class II MHC antigens in a sequence independent manner. Thus the functional region of class II-restricted epitopes is typically less than about 15 amino acid residues long. The size of linear B cell epitopes and the factors affecting their processing, like class II-restricted epitopes, are quite variable although such epitopes are frequently smaller in size than 15 amino acid residues. From the foregoing, it is advantageous, but not essential, that the size of the peptide is at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 amino acid residues. Suitably, the size of the peptide is no more than about 500, 200, 100, 80, 60, 50, 40 amino acid residues. In

certain advantageous embodiments, the size of the peptide is sufficient for presentation by an antigen-presenting cell of a T cell and/or a B cell epitope contained within the peptide.

[0132] Criteria for identifying and selecting effective antigenic peptides (*e.g.*, minimal peptide sequences capable of eliciting an immune response) can be found in the art. For example, Apostolopoulos *et al.,* (*Curr Opin Mol Ther*, 2000, 2: 29-36) discusses the strategy for identifying minimal antigenic peptide sequences based on an understanding of the three-dimensional structure of an antigen-presenting molecule and its interaction with both an antigenic peptide and T cell receptor. Shastri (*Curr Opin Immunol,* 1996, 8: 271-277) discloses how to distinguish rare peptides that serve to activate T cells from the thousands of peptides normally bound to MHC molecules.

2.3 Compositions

[0133] Exemplary compositions of the present invention include vaccines or constructs, including but not limited to recombinant vaccines.

[0134] In some embodiments, the immunostimulatory compositions have any one or more of the following characteristics:

[0135] (a) stimulate or induce an antigen-specific Th17 response (*e.g.,* an increase in cytokine expression of any one of IL-17A, IL-22, TNF-α, IL-17F, IL-21, IL-26, GM-CSF, and MIP-A);

[0136] (b) stimulate or induce an antigen-specific Th1 response (*e.g.*, of IFN-γ, IL-2 and TNF-β);

[0137] (c) suppress the expression of one or more of IL-13, IL-4, IL-5 and IL-9 by ILC2; and

[0138] (d) suppressing an Th2 immune response (*e.g.*, reduces stimulation of Th2 responses by conventional dendritic cells).

[0139] In some embodiments, the composition comprises a nucleic acid composition comprising: a first agent comprising a coding sequence for an immune stimulator operably linked to a regulatory polynucleotide, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject and a second agent comprising a coding sequence for an inhibitor of IL-25 function operably linked to a regulatory polynucleotide and from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible. The regulatory polynucleotide may be the same or different.

[0140] In some embodiments, the first and second polynucleotides are located on the same construct (or expression vector). In other embodiments, the first and second polynucleotides are located on different constructs.

[0141] The regulatory polynucleotide suitably comprises transcriptional and/or translational control sequences, which will be compatible for expression in the cell or tissue type of interest. Typically, the transcriptional and translational regulatory control sequences include, but are not limited to, a promoter sequence, a 5′ non-coding region, a *cis*-regulatory region such as a functional binding site for transcriptional regulatory protein or translational regulatory protein, an upstream open reading frame, ribosomal-binding sequences, transcriptional start site, translational start site, and/or nucleotide sequence which encodes a leader sequence, termination codon,

translational stop site and a 3′ non-translated region. Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. Promoter sequences contemplated by the present invention may be native to the organism of interest or may be derived from an alternative source, where the region is functional in the chosen organism. The choice of promoter will differ depending on the intended host. For example, promoters which could be used for expression in mammalian cells generally include the metallothionein promoter, which can be induced in response to heavy metals such as cadmium, the β-actin promoter as well as viral promoters such as the SV40 large T antigen promoter, human cytomegalovirus (CMV) immediate early (IE) promoter, rous sarcoma virus LTR promoter, adenovirus promoter, or a HPV promoter, particularly the HPV upstream regulatory region (URR) may also be used. All these promoters are well described and readily available in the art. Alternatively, the promoter may be lineage specific and, in this regard, epithelial-specific promoters are particularly desirable such as, but not limited to, promoters of the following genes transglutaminase type 1, involucrin, loricrin, SPR genes and filaggrin as well as those of keratin genes (*e.g.*, K10, K14, K5, K1).

[0142] The synthetic construct (or expression vector) may also comprise a 3′ non-translated sequence. A 3′ non-translated sequence refers to that portion of a gene comprising a DNA segment that contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is characterized by effecting the addition of polyadenylic acid tracts to the 3′ end of the mRNA precursor. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5′ AATAAA-3′ although variations are not uncommon. The 3′ non-translated regulatory DNA sequence preferably includes from about 50 to 1,000 nucleotide base pairs and may contain transcriptional and translational termination sequences in addition to a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression.

[0143] In some embodiments, the synthetic construct (or expression vector) further contains a screenable marker gene to permit identification of cells containing the synthetic construct. Screenable genes (*e.g., lacZ, gfp, etc.*) are well known in the art and will be compatible for expression in a particular cell or tissue type.

[0144] It will be understood, however, that expression of protein-encoding polynucleotides in heterologous systems is now well known, and the present invention is not directed to or dependent on any particular vector, transcriptional control sequence or technique for its production. Rather, synthetic polynucleotides prepared according to the methods as set forth herein may be introduced into selected cells or tissues or into a precursors or progenitors thereof in any suitable manner in conjunction with any suitable synthetic construct or vector, and the synthetic polynucleotides may be expressed with known promoters in any conventional manner.

[0145] The synthetic constructs or vectors can be introduced into suitable host cells for expression using any of a number of non-viral or viral gene delivery vectors. For example, retroviruses (in particular, lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful for gene therapy applications) can be inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*.

[0146] In one illustrative embodiment, retroviruses provide a convenient and effective platform for gene delivery systems. A selected nucleotide sequence that encodes an antigen corresponding to the target antigen and a selected nucleotide sequence that encodes an inhibitor of IL-25 function (where the two selected nucleotide sequences can be part of the same sequence or separate sequences) can be inserted into a construct or vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to a subject. Several illustrative retroviral systems have been described examples of which include: U.S. Patent No. 5,219,740; Miller and Rosman, *Bio Techniques*, 1989, 7: 980-990; Miller, A. D., *Human Gene Therapy*, 1990, 1: 5-14; Scarpa *et al.*, *Virology*, 1991, 180: 849-852; Burns *et al.*, *Proc Nat Acad Sci USA*, 1993, 90: 8033-8037; and Boris-Lawrie and Temin, *Cur Opin Genet Develop*, 1993, 3: 102-109.

[0147] In addition, several illustrative adenovirus-based systems have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (*see*, *e.g.*, Haj-Ahmad and Graham, *J Virol*, 1986, 57: 267-274; Bett *et al.*, *J Virol*, 1993, 67: 5911-5921; Mittereder *et al.*, *Human Gene Therapy,* 1994, 5: 717-729; Seth *et al.*, *J Virol*, 1994, 68: 933-940; Barr *et al.*, *Gene Therapy*, 1994, 1: 51-58; Berkner, K. L., *Bio Techniques*, 1988, 6: 616-629; and Rich *et al.*, *Human Gene Therapy*, 1993, 4: 461-476).

[0148] Various adeno-associated virus (AAV) vector systems have also been developed for polynucleotide delivery. AAV vectors can be readily constructed using techniques well known in the art. *See*, for example, U.S. patent nos. 5,173,414 and 5,139,941; International PCT publication nos. WO 92/01070 and WO 93/03769; Lebkowski *et al.*, *Molec Cell Biol*, 1988, 8: 3988-3996; Vincent *et al.*, *Vaccines*, 1990, 90, Cold Spring Harbor Laboratory Press; Carter, B. J., *Current Opinion in Biotechnology*, 1992, 3: 533-539; Muzyczka, N., *Current Topics in Microbiol and Immunol*, 1992 158: 97-129; Kotin, R. M., *Human Gene Therapy*, 1994, 5: 793-801; Shelling and Smith, *Gene Therapy*, 1: 165-169; and Zhou *et al.*, *J Exp Med*, 1994, 179: 1867-1875.

[0149] Additional viral vectors useful for delivering the antigen-encoding polynucleotide and the IL-25 inhibitor-encoding polynucleotide (which can be the same polynucleotide or two separate polynucleotides) by gene transfer include those derived from the pox family of viruses, such as vaccinia virus (*e.g.*, Modified Vaccinia Ankara (MVA) virus) and avian poxvirus (*e.g.*, Fowlpox virus). By way of example, vaccinia virus recombinants expressing the antigen-encoding polynucleotide and the IL-25 inhibitor-encoding polynucleotide can be constructed as follows. The polynucleotides are first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the expression products of interest into the viral genome. The resulting $TK^{(-)}$ recombinant can be selected by culturing the cells in the presence of 5-BrdU and picking viral plaques resistant thereto. In specific embodiments, a recombinant Fowlpox virus is prepared from which an antigen-encoding polynucleotide and an IL-4 antagonist-encoding polynucleotide are expressible for administration by a mucosal route (*e.g.*, intranasally) and a recombinant MVA virus is prepared from which an antigen-encoding polynucleotide and an IL-4 antagonist-encoding polynucleotide are expressible for administration by a systemic route (*e.g.*, intramuscularly). In illustrative examples of this type,

the antigen encoded by the antigen-encoding polynucleotide may comprise an HIV antigen and/or a *M. tuberculosis* antigen.

[0150] Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the coding sequences of interest. The use of an Avipox vector is particularly desirable in human and other mammalian species since members of the Avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant Avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses (*see*, *e.g.*, International PCT publication nos. WO 91/12882; WO 89/03429; and WO 92/03545).

[0151] Any of a number of alphavirus vectors can also be used for delivery of polynucleotide compositions of the present invention, such as those vectors described in U.S. patent nos. 5,843,723; 6,015,686; 6,008,035 and 6,015,694. Certain vectors based on Venezuelan Equine Encephalitis (VEE) can also be used, illustrative examples of which can be found in U.S. Pat. Nos. 5,505,947 and 5,643,576.

[0152] Moreover, molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael *et al.*, *J. Biol. Chem.* 268:6866-69, 1993; and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 89:6099-6103, 1992, can also be used for gene delivery under the invention.

[0153] In other illustrative embodiments, lentiviral vectors are employed to deliver an antigen-encoding polynucleotide into selected cells or tissues. Typically, these vectors comprise a 5′ lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3′ lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5′) or downstream (3′) of a coding sequence of interest (for example, a synthetic Gag or Env expression cassette of the present invention). A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV, BIV, EIAV, MVV, CAEV, and SIV. Illustrative examples of lentiviral vectors are described in International PCT Publication Nos. WO 00/66759, WO 00/00600, WO 99/24465, WO 98/51810, WO 99/51754, WO 99/31251, WO 99/30742, and WO 99/15641. Desirably, a third generation SIN lentivirus is used. Commercial suppliers of third generation SIN (self-inactivating) lentiviruses include INVITROGEN (VIRAPOWER Lentiviral Expression System). Detailed methods for construction, transfection, harvesting, and use of lentiviral vectors are given, for example, in the Invitrogen technical manual "ViraPower Lentiviral Expression System Version B 050102 25-0501." Lentiviral vectors have emerged as an efficient method for gene transfer. Improvements in biosafety characteristics have made these vectors suitable for use at biosafety level 2 (BL2). A number of safety features are incorporated into third generation SIN (self-inactivating) vectors. Deletion of the viral 3′ LTR U3 region results in a provirus that is unable to transcribe a full length viral RNA. In addition, a number of essential genes are provided in trans, yielding a viral stock that is capable of but a single round of infection and integration. Lentiviral vectors have several advantages, including: 1) pseudotyping of the vector using amphotropic envelope proteins allows them to infect virtually any cell type; 2) gene delivery to quiescent, post mitotic, differentiated cells, including neurones, has been demonstrated; 3) their low cellular toxicity is unique among transgene delivery systems; 4) viral integration into the genome permits long term transgene expression; 5) their packaging capacity (6-14 kb) is

much larger than other retroviral, or adeno-associated viral vectors. In a recent demonstration of the capabilities of this system, lentiviral vectors expressing GFP were used to infect murine stem cells resulting in live progeny, germline transmission, and promoter-, and tissue-specific expression of the reporter (*see*, Ailles, L. E. and Naldini, L., *Lentiviral Vectors*, Springer-Verlag, Berlin, Heidelberg, New York, 2002,31-52). An example of the current generation vectors is outlined in FIG. 2 of a review by Lois *et al.* (*see*, Lois, C., Hong, E.J., Pease, S., Brown, E.J., and Baltimore, D., *Science*, 2002, 295: 868-872).

[0154] In certain embodiments, a polynucleotide may be integrated into the genome of a target cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the polynucleotide may be stably maintained in the cell as a separate, episomal segment of DNA. Such polynucleotide segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. The manner in which the expression construct is delivered to a cell and where in the cell the polynucleotide remains is dependent on the type of expression construct employed.

[0155] In other embodiments, a polynucleotide is administered/delivered as "naked" DNA, for example as described in Ulmer *et al.*, *Science* 259:1745-49, 1993 and reviewed by Cohen, *Science* 259:1691-92, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

[0156] In still other embodiments, a composition of the present invention can be delivered *via* a particle bombardment approach, many of which have been described. In one illustrative example, gas-driven particle acceleration can be achieved with devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, Wis.), some examples of which are described in U.S. patent nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and European patent no. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand-held device, propelling the particles into a target tissue of interest.

[0157] In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, Oreg.), some examples of which are described in U.S. patent nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

2.3.1 Immune stimulating cell embodiments

[0158] The present invention also contemplates the use of cellular preparations comprising antigen-presenting cells, which present an antigen corresponding to at least a portion of the target antigen. Suitably, the antigen-presenting cells express or otherwise comprise the inhibitor of IL-25 function. Such antigen-presenting cells include professional or facultative antigen-presenting cells. Professional antigen-presenting cells function physiologically to present antigen in a form that is recognised by specific T cell receptors so as to stimulate or anergize a T lymphocyte or B lymphocyte mediated immune response. Professional antigen-presenting cells not only process and present antigens in the context of the major histocompatibility complex (MHC), but also

possess the additional immuno-regulatory molecules required to complete T cell activation or induce a tolerogenic response. Professional antigen-presenting cells include, but are not limited to, macrophages, monocytes, B lymphocytes, cells of myeloid lineage, including monocytic-granulocytic-DC precursors, marginal zone Kupffer cells, microglia, T cells, Langerhans cells and dendritic cells including interdigitating dendritic cells and follicular dendritic cells. Non-professional or facultative antigen-presenting cells typically lack one or more of the immunoregulatory molecules required to complete T lymphocyte activation or anergy. Examples of non-professional or facultative antigen-presenting cells include, but are not limited to, activated T lymphocytes, eosinophils, keratinocytes, astrocytes, follicular cells, microglial cells, thymic cortical cells, endothelial cells, Schwann cells, retinal pigment epithelial cells, myoblasts, vascular smooth muscle cells, chondrocytes, enterocytes, thymocytes, kidney tubule cells and fibroblasts. In some embodiments, the antigen-presenting cell is selected from monocytes, macrophages, B lymphocytes, cells of myeloid lineage, dendritic cells or Langerhans cells. In certain advantageous embodiments, the antigen-presenting cell expresses CD11c and includes a dendritic cell or a Langerhans cell.

[0159] Cellular preparations for stimulating an immune response to an antigen or group of antigens may be prepared according to any suitable method known to the skilled practitioner. Illustrative methods for preparing antigen-presenting cells for stimulating antigen-specific immune responses are described by Albert *et al.* (International Publication WO 99/42564), Takamizawa *et al.* (1997, *J Immunol*, 158(5): 2134-2142), Thomas and Lipsky (1994, *J Immunol*, 153(9):4016-4028), O'Doherty *et al.* (1994, *Immunology*, 82(3):487-93), Fearnley *et al.* (1997, *Blood*, 89(10): 3708-3716), Weissman *et al.* (1995, *Proc Natl Acad Sci U S A*, 92(3):826-830), Freudenthal and Steinman (1990, *Proc Natl Acad Sci U S A*, 87(19):7698-7702), Romani *et al.* (1996, *J Immunol Methods*, 196(2): 137-151), Reddy *et al.* (1997, *Blood*, 90(9):3640-3646), Thurnher *et al.* (1997, *Exp Hematol*, 25(3):232-237), Caux *et al.* (1996, *J Exp Med*, 184(2):695-706; 1996, *Blood*, 87(6):2376-85), Luft *et al.* (1998, *Exp Hematol*, 26(6):489-500; 1998, *J Immunol*, 161(4):1947-1953), Cella *et al.* (1999, *J Exp Med*, 189(5): 821-829;1997, *Nature*, 388(644):782-787; 1996, *J Exp Med*, 184(2):747-572), Ahonen *et al.* (1999, *Cell Immunol*, 197(1):62-72) and Piemonti *et al.* (1999, *J Immunol*, 162(11):6473-6481).

[0160] In some embodiments, antigen-presenting cells are isolated from a host, treated and then reintroduced or reinfused into the host. Conveniently, antigen-presenting cells can be obtained from the host to be treated either by surgical resection, biopsy, blood sampling, or other suitable technique. Such cells are referred to herein as "autologous" cells. In other embodiments, the antigen-presenting cells or cell lines are prepared and/or cultured from a different source than the host. Such cells are referred to herein as "allogeneic" cells. Desirably, allogeneic antigen-presenting cells or cell lines will share major and/or minor histocompatibility antigens to potential recipients (also referred to herein as 'generic' antigen-presenting cells or cell lines). In certain advantageous embodiments of this type, the generic antigen-presenting cells or cell lines comprise major histocompatibility (MHC) class I antigens compatible with a high percentage of the population (*i.e.*, at least 10, 20, 30, 40, 50, 60, 70, 75, 80, 85, 90, 92, 94 or 98%) that is susceptible or predisposed to a particular condition. Suitably, the generic antigen-presenting cells or cell lines naturally express an immunostimulatory molecule as described herein, especially an immunostimulatory membrane molecule, at levels sufficient to trigger an immune response, desirably a T lymphocyte immune response (*e.g.*, a cytotoxic T lymphocyte immune response), in

the intended host. In certain embodiments, the antigen-presenting cells or cell lines are highly susceptible to treatment with at least one IFN as described in International PCT publication no. WO 01/88097 (*i.e.*, implied high-level expression of class I HLA).

[0161] In some embodiments, antigen-presenting cells are made antigen-specific by a process including contacting or 'pulsing' the antigen-presenting cells with an antigen that corresponds to at least a portion of the target antigen for a time and under conditions sufficient to permit the antigen to be internalized by the antigen-presenting cells; and culturing the antigen-presenting cells so contacted for a time and under conditions sufficient for the antigen to be processed for presentation by the antigen-presenting cells. The pulsed cells can then be used to stimulate autologous or allogeneic T cells *in vitro* or *in vivo*. The amount of antigen to be placed in contact with antigen-presenting cells can be determined empirically by persons of skill in the art. Typically antigen-presenting cells are incubated with antigen for about 1 to 6 hours at 37°C. Usually, for purified antigens and peptides, 0.1-10 μg/mL is suitable for producing antigen-specific antigen-presenting cells. The antigen should be exposed to the antigen-presenting cells for a period of time sufficient for those cells to internalize the antigen. The time and dose of antigen necessary for the cells to internalize and present the processed antigen may be determined using pulse-chase protocols in which exposure to antigen is followed by a washout period and exposure to a read-out system *e.g.*, antigen reactive T cells. Once the optimal time and dose necessary for cells to express processed antigen on their surface is determined, a protocol may be used to prepare cells and antigen for inducing tolerogenic responses. Those of skill in the art will recognize in this regard that the length of time necessary for an antigen-presenting cell to present an antigen may vary depending on the antigen or form of antigen employed, its dose, and the antigen-presenting cell employed, as well as the conditions under which antigen loading is undertaken. These parameters can be determined by the skilled artisan using routine procedures.

[0162] The delivery of exogenous antigen to an antigen-presenting cell can be enhanced by methods known to practitioners in the art. For example, several different strategies have been developed for delivery of exogenous antigen to the endogenous processing pathway of antigen-presenting cells, especially dendritic cells. These methods include insertion of antigen into pH-sensitive liposomes (Zhou and Huang, 1994, *Immunomethods*, 4:229-235), osmotic lysis of pinosomes after pinocytic uptake of soluble antigen (Moore *et al.*, 1988, *Cell*, 54:777-785), coupling of antigens to potent adjuvants (Aichele *et al.*, 1990, *J. Exp. Med.*, 171: 1815-1820; Gao *et al.*, 1991, *J. Immunol.*, 147: 3268-3273; Schulz *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88: 991-993; Kuzu *et al.*, 1993, *Euro. J. Immunol.*, 23: 1397-1400; and Jondal *et al.*, 1996, *Immunity* 5: 295-302) and apoptotic cell delivery of antigen (Albert *et al.* 1998, *Nature* 392:86-89; Albert *et al.* 1998, *Nature Med.* 4:1321-1324; and in International Publications WO 99/42564 and WO 01/85207). Recombinant bacteria (*e.g.*, *E. coli*) or transfected host mammalian cells may be pulsed onto dendritic cells (as particulate antigen, or apoptotic bodies respectively) for antigen delivery. Recombinant chimeric virus-like particles (VLPs) have also been used as vehicles for delivery of exogenous heterologous antigen to the MHC class I processing pathway of a dendritic cell line (Bachmann *et al.*, 1996, *Eur. J. Immunol.*, 26(11): 2595-2600).

[0163] Alternatively, or in addition, an antigen may be linked to, or otherwise associated with, a cytolysin to enhance the transfer of the antigen into the cytosol of an antigen-presenting cell of the invention for delivery to the MHC class I pathway. Exemplary cytolysins

include saponin compounds such as saponin-containing Immune Stimulating Complexes (ISCOMs) (see *e.g.*, Cox and Coulter, 1997, *Vaccine* 15(3): 248-256 and U.S. Patent No. 6,352,697), phospholipases (see, *e.g.*, Camilli *et al.*, 1991, *J. Exp. Med.* 173: 751-754), pore-forming toxins *(e.g.*, an α-toxin), natural cytolysins of gram-positive bacteria, such as listeriolysin O (LLO, *e.g.*, Mengaud *et al.*, 1988, *Infect. Immun.* 56: 766-772 and Portnoy *et al.*, 1992, *Infect. Immun.* 60: 2710-2717), streptolysin O (SLO, *e.g.*, Palmer *et al.*, 1998, *Biochemistry* 37(8): 2378-2383) and perfringolysin O (PFO, *e.g.*, Rossjohn *et al.*, *Cell* 89(5): 685-692). Where the antigen-presenting cell is phagosomal, acid activated cytolysins may be advantageously used. For example, listeriolysin exhibits greater pore-forming ability at mildly acidic pH (the pH conditions within the phagosome), thereby facilitating delivery of vacuole (including phagosome and endosome) contents to the cytoplasm (see, *e.g.*, Portnoy *et al.*, *Infect. Immun.* 1992, 60: 2710-2717).

[0164] The cytolysin may be provided together with a pre-selected antigen in the form of a single composition or may be provided as a separate composition, for contacting the antigen-presenting cells. In one embodiment, the cytolysin is fused or otherwise linked to the antigen, wherein the fusion or linkage permits the delivery of the antigen to the cytosol of the target cell. In another embodiment, the cytolysin and antigen are provided in the form of a delivery vehicle such as, but not limited to, a liposome or a microbial delivery vehicle selected from virus, bacterium, or yeast. Suitably, when the delivery vehicle is a microbial delivery vehicle, the delivery vehicle is non-virulent. In a preferred embodiment of this type, the delivery vehicle is a non-virulent bacterium, as for example described by Portnoy *et al.* in U.S. Patent No. 6,287,556, comprising a first polynucleotide encoding a non-secreted functional cytolysin operably linked to a regulatory polynucleotide which expresses the cytolysin in the bacterium, and a second polynucleotide encoding one or more pre-selected antigens. Non-secreted cytolysins may be provided by various mechanisms, *e.g.*, absence of a functional signal sequence, a secretion incompetent microbe, such as microbes having genetic lesions (*e.g.*, a functional signal sequence mutation), or poisoned microbes, *etc*. A wide variety of nonvirulent, non-pathogenic bacteria may be used; preferred microbes are relatively well characterized strains, particularly laboratory strains of *E. coli*, such as MC4100, MC1061, DH5α, *etc*. Other bacteria that can be engineered for the invention include well-characterized, nonvirulent, non-pathogenic strains of *Listeria monocytogenes*, *Shigella flexneri*, mycobacterium, *Salmonella*, *Bacillus subtilis*, *etc*. In particular embodiments, the bacteria are attenuated to be non-replicative, non-integrative into the host cell genome, and/or non-motile inter- or intra-cellularly.

[0165] The delivery vehicles described above can be used to deliver one or more antigens to virtually any antigen-presenting cell capable of endocytosis of the subject vehicle, including phagocytic and non-phagocytic antigen-presenting cells. In embodiments when the delivery vehicle is a microbe, the subject methods generally require microbial uptake by the target cell and subsequent lysis within the antigen-presenting cell vacuole (including phagosomes and endosomes).

[0166] In some other embodiments, in order to enhance the class I presentation of the antigen, the immune stimulator is modified to comprise an intracellular degradation signal or degron. The degron is suitably a ubiquitin-mediated degradation signal selected from a destabilizing amino acid at the amino-terminus of an antigen, a ubiquitin acceptor, a ubiquitin or combination thereof.

[0167] Thus, in one embodiment, the immune stimulator is modified to include a destabilizing amino acid at its amino-terminus so that the protein so modified is subject to the N-end rule pathway as disclosed, for example, by Bachmair *et al.*, in U.S. Pat. No. 5,093,242 and by Varshavsky *et al.*, in U.S. Pat. No. 5,122,463. In a preferred embodiment of this type, the destabilizing amino acid is selected from isoleucine and glutamic acid, more preferably from histidine tyrosine and glutamine, and even more preferably from aspartic acid, asparagine, phenylalanine, leucine, tryptophan and lysine. In an especially preferred embodiment, the destabilizing amino acid is arginine.

[0168] Modification or design of the amino-terminus of a protein can also be accomplished at the genetic level. Conventional techniques of site-directed mutagenesis for addition or substitution of appropriate codons to the 5′ end of an isolated or synthesized antigen-encoding polynucleotide can be employed to provide a desired amino-terminal structure for the encoded protein. For example, so that the protein expressed has the desired amino acid at its amino-terminus the appropriate codon for a destabilizing amino acid can be inserted or built into the amino-terminus of the protein-encoding sequence. Where necessary, a nucleic acid sequence encoding the amino-terminal region of a protein can be modified to introduce one or more lysine residues in an appropriate context, which act as a ubiquitin acceptor as described in more detail below. This can be achieved most conveniently by employing DNA constructs encoding "universal destabilizing segments". A universal destabilizing segment comprises a nucleic acid construct which encodes a polypeptide structure, preferably segmentally mobile, containing one or more lysine residues, the codons for lysine residues being positioned within the construct such that when the construct is inserted into the coding sequence of the antigen-encoding polynucleotide, the lysine residues are sufficiently spatially proximate to the amino-terminus of the encoded protein to serve as the second determinant of the complete amino-terminal degradation signal. The insertion of such constructs into the 5′ portion of an antigen-encoding polynucleotide would provide the encoded protein with a lysine residue (or residues) in an appropriate context for destabilization.

[0169] The codon for the amino-terminal amino acid of the protein of interest can be made to encode the desired amino acid by, for example, site-directed mutagenesis techniques currently standard in the field. Suitable mutagenesis methods are described for example in the relevant sections of Ausubel, *et al.* (*supra*) and of Sambrook, *et al.*, (*supra*). Alternatively, suitable methods for altering DNA are set forth, for example, in U.S. Pat. Nos. 4,184,917, 4,321,365 and 4,351,901, which are incorporated herein by reference. Instead of *in vitro* mutagenesis, the synthetic polynucleotide can be synthesized *de novo* using readily available machinery. Sequential synthesis of DNA is described, for example, in U.S. Pat. No. 4,293,652. However, it should be noted that the present invention is not dependent on, and not directed to, any one particular technique for constructing a polynucleotide encoding a modified antigen as described herein.

[0170] If the antigen-encoding polynucleotide is a synthetic or recombinant polynucleotide the appropriate 5′ codon can be built-in during the synthetic process. Alternatively, nucleotides for a specific codon can be added to the 5′ end of an isolated or synthesized polynucleotide by ligation of an appropriate nucleic acid sequence to the 5′ (amino-terminus-encoding) end of the polynucleotide. Nucleic acid inserts encoding appropriately located lysine residues (such as the "universal destabilizing segments" described above) can suitably be inserted

into the 5′ region to provide for the second determinant of the complete amino-terminal degradation.

[0171] In a preferred embodiment, the modified antigen, which comprises a destabilizing amino acid at its amino terminus, is fused or otherwise conjugated to a masking entity, which masks said amino terminus so that when unmasked the antigen will exhibit the desired rate of intracellular proteolytic degradation. Suitably, the masking entity is a masking protein sequence. The fusion protein is designed so that the masking protein sequence fused to the amino-terminus of the protein of interest is susceptible to specific cleavage at the junction between the two. Removal of the protein sequence thus unmasks the amino-terminus of the protein of interest and the half-life of the released protein is thus governed by the predesigned amino-terminus. The fusion protein can be designed for specific cleavage *in vivo*, for example, by a host cell endoprotease or for specific cleavage in an *in vitro* system where it can be cleaved after isolation from a producer cell (which lacks the capability to cleave the fusion protein). Thus, in a preferred embodiment, the masking protein sequence is cleavable by an endoprotease, which is preferably an endogenous endoprotease of a mammalian cell. Suitable endoproteases include, but are not restricted to, serine endoproteases (*e.g.*, subtilisins and furins) as described, for example, by Creemers, *et al.* (1998, *Semin. Cell Dev. Biol.* 9 (1): 3-10), proteasomal endopeptidases as described, for example, by Zwickl, *et al.* (2000, *Curr. Opin. Struct. Biol.* 10 (2): 242-250), proteases relating to the MHC class I processing pathway as described, for example, by Stolze *et al.* (2000, *Nat. Immunol.* 1 413-418) and signal peptidases as described, for example, by Dalbey, *et al.* (1997, *Protein Sci.* 6 (6): 1129-1138). In a preferred embodiment of this type, the masking protein sequence comprises a signal peptide sequence. Suitable signal peptides sequences are described, for example, by Nothwehr *et al.* (1990,. *Bioessays* 12 (10): 479-484), Izard, *et al.* (1994, *Mol. Microbiol.* 13 (5): 765-773), Menne, *et al.* (2000, *Bioinformatics.* 16 (8): 741-742) and Ladunga (2000, *Curr. Opin. Biotechnol.* 11 (1): 13-18). Suitably, an endoprotease cleavage site is interposed between the masking protein sequence and the antigen.

[0172] A modified antigen with an attached masking sequence may be conveniently prepared by fusing a nucleic acid sequence encoding a masking protein sequence upstream of another nucleic acid sequence encoding an antigen, which corresponds to the target antigen of interest and which includes a destabilizing amino acid at its amino-terminus. The codon for the amino-terminal amino acid of the antigen of interest is suitably located immediately adjacent to the 3′ end of the masking protein-encoding nucleic acid sequence.

[0173] In another embodiment, the antigen is modified to include, or is otherwise associated with, a ubiquitin acceptor which is a molecule that preferably contains at least one residue appropriately positioned from the N-terminal of the antigen as to be able to be bound by ubiquitin molecules. Such residues preferentially have an epsilon amino group such as lysine. Physical analysis demonstrates that multiple lysine residues function as ubiquitin acceptor sites (King *et al.*, 1996, *Mol. Biol. Cell* 7: 1343-1357; King *et al.*, 1996, *Science* 274: 1652-1659). Examples of other ubiquitin acceptors include lacI or Sindis virus RNA polymerase. Ubiquitination at the N-terminal of the protein specifically targets the protein for degradation *via* the ubiquitin-proteosome pathway.

[0174] Other protein processing signals that destabilize an antigen of interest and allow for enhanced intracellular degradation are contemplated in the present invention. These other

methods may not necessarily be mediated by the ubiquitin pathway, but may otherwise permit degradation of proteins in the cytoplasm *via* proteasomes. For example, the present invention contemplates the use of other intracellular processing signals which govern the rate(s) of intracellular protein degradation including, but not limited to, those described by Bohley *et al.* (1996, *Biol. Chem. Hoppe. Seyler* 377: 425-435). Such processing signals include those that allow for phosphorylation of the target protein (Yaglom *et al.*, 1996, *Mol. Cell Biol.* 16: 3679-3684; Yaglom *et al.*, 1995, *Mol. Cell Biol.* 15: 731-741). Also contemplated by the present invention are modification of an parent antigen that allow for post-translational arginylation (Ferber *et al.* 1987, *Nature* 326: 808-811; Bohley *et al.*, 1991, *Biomed. Biochim. Acta* 50: 343-346) of the protein which can enhance its rate(s) of intracellular degradation. The present invention also contemplates the use of certain structural features of proteins that can influence higher rates of intracellular protein turn-over, including protein surface hydrophobicity, clusters of hydrophobic residues within the protein (Sadis *et al.*, *Mol Cell Biol*, 1995, 15: 4086-4094), certain hydrophobic pentapeptide motifs at the protein's carboxy-terminus (C-terminus) (*e.g.*, ARINV), as found on the C-terminus of ornithine decarboxylase (Ghoda *et al.*, *Mol Cell Biol*, 1992, 12: 2178-2185; Li, *et al.*, *Mol Cell Biol,* 1994, 14: 87-92), or AANDENYALAA, as found in C-terminal tags of aberrant polypeptides (Keiler *et al.*, *Science*, 1996, 271: 990-993,) or PEST regions (regions rich in proline (P), glutamic acid (E), serine (S), and threonine (T), which are optionally flanked by amino acids comprising electropositive side chains (Rogers *et al.*, *Science,* 1986, 234 (4774): 364-368; 1988, *J. Biol. Chem.* 263: 19833-19842). Moreover, certain motifs have been identified in proteins that appear necessary and possibly sufficient for achieving rapid intracellular degradation. Such motifs include RXALGXIXN region (where X = any amino acid) in cyclins (Glotzer *et al., Nature*, 1991, 349: 132-138) and the KTKRNYSARD motif in isocitrate lyase (Ordiz *et al.*, *FEBS Lett*, 1996, 385: 43-46).

[0175] The present invention also contemplates enhanced cellular degradation of a parent antigen which may occur by the incorporation into that antigen of known protease cleavage sites. For example, amyloid beta-protein can be cleaved by beta- and gamma-secretase (Iizuka *et al.*, 1996, *Biochem. Biophys. Res. Commun.* 218: 238-242) and the two-chain vitamin K-dependent coagulation factor X can be cleaved by calcium-dependent endoprotease(s) in liver (Wallin *et al.*, 1994, *Thromb. Res.* 73: 395-403).

[0176] In yet another embodiment, the parent antigen is conjugated to a ubiquitin or a biologically active fragment thereof, to produce a modified antigen whose rate of intracellular proteolytic degradation is increased, enhanced or otherwise elevated relative to the parent antigen. In a preferred embodiment of this type, the ubiquitin or biologically active fragment is fused, or otherwise conjugated, to the antigen. Suitably, the ubiquitin is of mammalian origin, more preferably of human or other primate origin.

[0177] In one embodiment, the ubiquitin-antigen fusion protein is suitably produced by covalently attaching an antigen corresponding to the target antigen to a ubiquitin or a biologically active fragment thereof. Covalent attachment may be effected by any suitable means known to persons of skill in the art. For example, protein conjugates may be prepared by linking proteins together using bifunctional reagents. The bifunctional reagents can be homobifunctional or heterobifunctional.

[0178] Homobifunctional reagents are molecules with at least two identical functional groups. The functional groups of the reagent generally react with one of the functional groups on a

protein, typically an amino group. Examples of homobifunctional reagents include glutaraldehyde and diimidates. An example of the use of glutaraldehyde as a cross-linking agent is described by Poznansky *et al.* (1984, *Science*, 223: 1304-1306). The use of diimidates as a cross-linking agent is described for example by Wang, *et al.* (1977, *Biochemistry*, 16: 2937-2941). Although it is possible to use homobifunctional reagents for the purpose of forming a modified antigen according to the invention, skilled practitioners in the art will appreciate that it is difficult to attach different proteins in an ordered fashion with these reagents. In this regard, in attempting to link a first protein with a second protein by means of a homobifunctional reagent, one cannot prevent the linking of the first protein to each other and of the second to each other. Heterobifunctional crosslinking reagents are, therefore, preferred because one can control the sequence of reactions, and combine proteins at will. Heterobifunctional reagents thus provide a more sophisticated method for linking two proteins. These reagents require one of the molecules to be joined, hereafter called Partner B, to possess a reactive group not found on the other, hereafter called Partner A, or else require that one of the two functional groups be blocked or otherwise greatly reduced in reactivity while the other group is reacted with Partner A. In a typical two-step process for forming heteroconjugates, Partner A is reacted with the heterobifunctional reagent to form a derivatized Partner A molecule. If the unreacted functional group of the cross linker is blocked, it is then deprotected. After deprotection, Partner B is coupled to derivatized Partner A to form the conjugate. Primary amino groups on Partner A are reacted with an activated carboxylate or imidate group on the cross linker in the derivatization step. A reactive thiol or a blocked and activated thiol at the other end of the cross linker is reacted with an electrophilic group or with a reactive thiol, respectively, on Partner B. When the cross linker possesses a reactive thiol, the electrophile on Partner B preferably will be a blocked and activated thiol, a maleimide, or a halomethylene carbonyl (*e.g.*, bromoacetyl or iodoacetyl) group. Because biological macromolecules do not naturally contain such electrophiles, they must be added to Partner B by a separate derivatization reaction. When the cross linker possesses a blocked and activated thiol, the thiol on Partner B with which it reacts may be native to Partner B.

[0179] An example of a heterobifunctional reagent is N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (see for example Carlsson *et al.,* 1978, *Biochem. J.*, 173: 723-737). Other heterobifunctional reagents for linking proteins include for example succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) (Yoshitake *et al.*, 1979, *Eur. J. Biochem*, 101: 395-399), 2-iminothiolane (IT) (Jue *et al.*, 1978, *Biochemistry*, 17: 5399-5406), and S-acetyl mercaptosuccinic anhydride (SAMSA) (Klotz and Heiney, 1962, *Arch. Biochem. Biophys.*, 96: 605-612). All three react preferentially with primary amines (*e.g.*, lysine side chains) to form an amide or amidine group which links a thiol to the derivatized molecule (*e.g.*, a heterologous antigen) *via* a connecting short spacer arm, one to three carbon atoms long. Examples of heterobifunctional reagents comprising reactive groups having a double bond that reacts with a thiol group include SMCC mentioned above, succinimidyl m-maleimidobenzoate, succinimidyl 3-(maleimido)propionate, sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, sulfosuccinimidyl 4-(N-maleimidomethylcyclohexane-1-carboxylate and maleimidobenzoyl-N-hydroxysuccinimide ester (MBS). In a preferred embodiment, MBS is used to produce the conjugate. Other heterobifunctional reagents for forming conjugates of two proteins are described for example by Rodwell *et al.* in U.S. Pat. No. 4,671,958 and by Moreland *et al.* in U.S. Pat. No. 5,241,078.

[0180] In an alternate embodiment, a ubiquitin-antigen fusion protein is suitably expressed by a synthetic chimeric polynucleotide comprising a first nucleic acid sequence, which encodes an antigen corresponding to the target antigen, and which is linked downstream of, and in reading frame with, a second nucleic acid sequence encoding a ubiquitin or biologically active fragment thereof. In a preferred embodiment of this type, the second polynucleotide comprises a first nucleic acid sequence, which encodes an antigen corresponding to the target antigen, and which is linked immediately adjacent to, downstream of, and in reading frame with, a second nucleic acid sequence encoding a ubiquitin or biologically active fragment thereof. In another embodiment, the second polynucleotide comprises a first nucleic acid sequence, which encodes an antigen corresponding to the target antigen, and which is linked upstream of, and in reading frame with, a second nucleic acid sequence encoding a ubiquitin or biologically active fragment thereof. In yet another embodiment of this type, the second polynucleotide comprises a first nucleic acid sequence, which encodes an antigen corresponding to the target antigen, and which is linked immediately adjacent to, upstream of, and in reading frame with, a second nucleic acid sequence encoding a ubiquitin or biologically active fragment thereof.

[0181] In other embodiments, the antigen is produced inside the antigen-presenting cell by introduction of a suitable expression vector as for example described above. The antigen-encoding portion of the expression vector may comprise a naturally-occurring sequence or a variant thereof, which has been engineered using recombinant techniques. In one example of a variant, the codon composition of an antigen-encoding polynucleotide is modified to permit enhanced expression of the antigen in a target cell or tissue of choice using methods as set forth in detail in International Publications WO 99/02694 and WO 00/42215. Briefly, these methods are based on the observation that translational efficiencies of different codons vary between different cells or tissues and that these differences can be exploited, together with codon composition of a gene, to regulate expression of a protein in a particular cell or tissue type. Thus, for the construction of codon-optimized polynucleotides, at least one existing codon of a parent polynucleotide is replaced with a synonymous codon that has a higher translational efficiency in a target cell or tissue than the existing codon it replaces. Although it is preferable to replace all the existing codons of a parent nucleic acid molecule with synonymous codons which have that higher translational efficiency, this is not necessary because increased expression can be accomplished even with partial replacement. Suitably, the replacement step affects 5, 10, 15, 20, 25, 30%, more preferably 35, 40, 50, 60, 70% or more of the existing codons of a parent polynucleotide.

[0182] The expression vector for introduction into the antigen-presenting cell will be compatible therewith such that the antigen-encoding polynucleotide is expressible by the cell. For example, expression vectors of this type can be derived from viral DNA sequences including, but not limited to, adenovirus, adeno-associated viruses, herpes-simplex viruses and retroviruses such as B, C, and D retroviruses as well as spumaviruses and modified lentiviruses. Suitable expression vectors for transfection of animal cells are described, for example, by Wu and Ataai (*Curr Opin Biotechnol*, 2000, 11(2): 205-208), Vigna and Naldini (*J Gene Med,* 2000, 2(5): 308-316), Kay, *et al.* (*Nat Med*, 2001, 7(1): 33-40), Athanasopoulos, *et al.* (*Int J Mol Med*, 2000, 6(4): 363-375) and Walther and Stein (*Drugs*, 2000, 60(2): 249-271). The expression vector is introduced into the antigen-presenting cell by any suitable means which will be dependent on the particular choice of expression vector and antigen-presenting cell employed. Such means of introduction are well-known to those skilled in the art. For example, introduction can be effected by use of contacting

(*e.g.*, in the case of viral vectors), electroporation, transformation, transduction, conjugation or triparental mating, transfection, infection membrane fusion with cationic lipids, high-velocity bombardment with DNA-coated microprojectiles, incubation with calcium phosphate-DNA precipitate, direct microinjection into single cells, and the like. Other methods also are available and are known to those skilled in the art. Alternatively, the vectors are introduced by means of cationic lipids, *e.g.*, liposomes. Such liposomes are commercially available (*e.g.*, LIPOFECTIN®, LIPOFECTAMINE™, and the like, supplied by Life Technologies, Gibco BRL, Gaithersburg, Md.). It will be understood by persons of skill in the art that the techniques for assembling and expressing antigen-encoding nucleic acid molecules, and/or immune stimulator encoding nucleic acid molecules as described herein *e.g.*, synthesis of oligonucleotides, nucleic acid amplification techniques, transforming cells, constructing vectors, expressions system and the like and transducing or otherwise introducing nucleic acid molecules into cells are well established in the art, and most practitioners are familiar with the standard resource materials for specific conditions and procedures.

[0183] In some embodiments, the antigen-specific antigen-presenting cells are obtained by isolating antigen-presenting cells or their precursors from a cell population or tissue to which modification of an immune response is desired. Typically, some of the isolated antigen-presenting cells or precursors will constitutively present antigens or have taken up such antigen *in vivo* that are targets or potential targets of an immune response for which stimulation or inhibition of an immune response is desired. In this instance, the delivery of exogenous antigen is not essential. Alternatively, cells may be derived from biopsies of healthy or diseased tissues, lysed or rendered apoptotic and the pulsed onto antigen-presenting cells (*e.g.*, dendritic cells). In certain embodiments of this type, the antigen-presenting cells represent cancer or tumor cells to which an antigen-specific immune response is required. Illustrative examples of cancers or tumor cells include cells of sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocyte) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In certain embodiments, the cancer or tumor cells are selected from the group consisting of melanoma cells and mammary carcinoma cells.

[0184] In some of the above embodiments, the cancer or tumor cells will constitute facultative or non-professional antigen-presenting cells, and may in some instances require further modification to enhance their antigen-presenting functions. In these instances, the antigen-

presenting cells are further modified to express one or more immunoregulatory molecules, which include any molecules occurring naturally in animals that may regulate or directly influence immune responses including: proteins involved in antigen processing and presentation such as TAP1/TAP2 transporter proteins, proteosome molecules such as LMP2 and LMP7, heat shock proteins such as gp96, HSP70 and HSP90, and major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules; factors that provide co-stimulation signals for T cell activation such as B7 and CD40; factors that provide co-inhibitory signals for direct killing of T cells or induction of T lymphocyte or B lymphocyte anergy or stimulation of T regulatory cell (Treg) generation such as OX-2, programmed death-1 ligand (PD-1L); accessory molecules such as CD83; chemokines; lymphokines and cytokines such as IFN s α, β and γ, interleukins (*e.g.*, IL-2, IL-7, IL-12, IL-15, IL-22, *etc.*), factors stimulating cell growth (*e.g.*, GM-SCF) and other factors (*e.g.*, tumor necrosis factors (TNFs), DC-SIGN, MIP1α, MIP1β and transforming growth factor-β (TGF-β). In certain advantageous embodiments, the immunoregulatory molecules are selected from a B7 molecule (*e.g.*, B7-1, B7-2 or B7-3) and an ICAM molecule (*e.g.*, ICAM-1 and ICAM-2).

[0185] Instead of recombinantly expressing immunoregulatory molecules, antigen-presenting cells expressing the desired immunostimulatory molecule(s) may be isolated or selected from a heterogeneous population of cells. Any method of isolation/selection is contemplated by the present invention, examples of which are known to those of skill in the art. For instance, one can take advantage of one or more particular characteristics of a cell to specifically isolate that cell from a heterogeneous population. Such characteristics include, but are not limited to, anatomical location of a cell, cell density, cell size, cell morphology, cellular metabolic activity, cell uptake of ions such as Ca^{2+}, K^{+}, and H^{+} ions, cell uptake of compounds such as stains, markers expressed on the cell surface, protein fluorescence, and membrane potential. Suitable methods that can be used in this regard include surgical removal of tissue, flow cytometry techniques such as fluorescence-activated cell sorting (FACS), immunoaffinity separation (*e.g.*, magnetic bead separation such as DYNABEAD™ separation), density separation (*e.g.*, metrizamide, PERCOLL™, or FICOLL™ gradient centrifugation), and cell-type specific density separation. Desirably, the cells are isolated by flow cytometry or by immunoaffinity separation using an antigen-binding molecule that is immuno-interactive with the immunoregulatory molecule.

[0186] Alternatively, the immunoregulatory molecule can be provided to the antigen-presenting cells in soluble form. In some embodiments of this type, the immunoregulatory molecule is a B7 molecule that lacks a functional transmembrane domain (*e.g.*, that comprises a B7 extracellular domain), non-limiting examples of which are described by McHugh *et al.* (*Clin Immunol Immunopathol*, 1998, 87(1): 50-59), Faas *et al.* (*J Immunol*, 2000, 164(12): 6340-6348) and Jeannin *et al.* (*Immunity*, 2000, 13(3): 303-312). In other embodiments of this type, the immunostimulatory protein is a B7 derivative including, but not limited to, a chimeric or fusion protein comprising a B7 molecule, or biologically active fragment thereof, or variant or derivative of these, linked together with an antigen-binding molecule such as an immunoglobulin molecule or biologically active fragment thereof. For example, a polynucleotide encoding the amino acid sequence corresponding to the extracellular domain of the B7-1 molecule, containing amino acids from about position 1 to about position 215, is joined to a polynucleotide encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human Ig Cγ1, using PCR, to form a polynucleotide that is expressed as a B7Ig fusion protein. DNA encoding the amino acid sequence

corresponding to a B7Ig fusion protein has been deposited with the American Type culture Collection (ATCC) in Rockville, Md., under the Budapest Treaty on May 31, 1991 and accorded accession number 68627. Techniques for making and assembling such B7 derivatives are disclosed for example by Linsley *et al.* (U.S. Pat. No. 5,580,756). Reference also may be made to Sturmhoefel *et al.* (*Cancer Res*, 1999, 59: 4964-4972) who disclose fusion proteins comprising the extracellular region of B7-1 or B7-2 fused in frame to the Fc portion of IgG2a.

[0187] The half-life of a soluble immunoregulatory molecule may be prolonged by any suitable procedure if desired. Preferably, such molecules are chemically modified with polyethylene glycol (PEG), including monomethoxy-polyethylene glycol, as for example disclosed by Chapman *et al.* (*Nature Biotechnology*, 1999, 17: 780-783).

[0188] Alternatively, or in addition, the antigen-presenting cells are cultured in the presence of at least one IFN for a time and under conditions sufficient to enhance the antigen presenting function of the cells and washing the cells to remove the IFN(s). In certain advantageous embodiments of this type, the step of culturing may comprise contacting the cells with at least one type I IFN and/or a type II IFN. The at least one type I IFN is suitably selected from the group consisting of an IFN-α, an IFN-β, a biologically active fragment of an IFN-α, a biologically active fragment of an IFN-β, a variant of an IFN-α, a variant of an IFN-β, a variant of a said biologically active fragment, a derivative of an IFN-α, a derivative of an IFN-β, a derivative of a said biologically active fragment, a derivative of a said variant, an analogue of IFN-α and an analogue of IFN-β. Typically, the type II IFN is selected from the group consisting of an IFN-γ, a biologically active fragment of an IFN-γ, a variant of an IFN-γ, a variant of said biologically active fragment, a derivative of an IFN-γ, a derivative of said biologically active fragment, a derivative of said variant and an analogue of an IFN-γ. Exemplary methods and conditions for enhancing the antigen-presenting functions of antigen-presenting cells using IFN treatment are described in International PCT publication no. WO 2001/88097.

[0189] In some embodiments, the antigen-presenting cells (*e.g.*, cancer cells) or cell lines are suitably rendered inactive to prevent further proliferation once administered to the subject. Any physical, chemical, or biological means of inactivation may be used, including but not limited to irradiation (generally with at least about 5,000 cGy, usually at least about 10,000 cGy, typically at least about 20,000 cGy); or treatment with mitomycin-C (usually at least 10 μg/mL; more usually at least about 50 μg /mL).

[0190] The antigen-presenting cells may be obtained or prepared to contain and/or express one or more antigens by any number of means, such that the antigen(s) or processed form(s) thereof, is (are) presented by those cells for potential modulation of other immune cells, including T lymphocytes and B lymphocytes, and particularly for producing T lymphocytes and B lymphocytes that are primed to respond to a specified antigen or group of antigens.

[0191] The present invention also contemplates co-introducing an agent that comprises an inhibitor of IL-13 function, and/or an inhibitor of IL-4 function into an antigen-presenting cell or antigen-presenting cell precursor so that the antigen-present cell co-expresses or co-presents both the antigen and the inhibitor of IL-13 function and/or the inhibitor of IL-4 function.

[0192] The agents of the present invention may be encapsulated, adsorbed to, or associated with, particulate carriers. Such carriers can be used to selectively introduce the agents

to cells of the immune system. The particles can be taken up by professional antigen presenting cells such as macrophages and dendritic cells, and/or can enhance antigen presentation through other mechanisms such as stimulation of cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, *e.g.*, Jeffery *et al.*, 1993, Pharm. Res. 10:362-368; McGee J. P., *et al.*, 1997, J Microencapsul. 14(2):197-210; O'Hagan D. T., *et al.*, 1993, Vaccine 11(2):149-54.

[0193] Furthermore, other particulate systems and polymers can be used for the in vivo delivery of the agents of the present invention. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like, will find use with the present methods. See, *e.g.*, Felgner, P. L., Advanced Drug Delivery Reviews (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R. N., *et al.*, U.S. Pat. No. 5,831,005, issued Nov. 3, 1998) may also be used for delivery of a nucleic acid construct of the present invention.

[0194] Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering agents that are in nucleic acid form (*e.g.*, nucleic acid constructs of the present invention). The particles are coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefor, see, *e.g.*, U.S. Pat. Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. In illustrative examples, gas-driven particle acceleration can be achieved with devices such as those manufactured by PowderMed Pharmaceuticals PLC (Oxford, UK) and PowderMed Vaccines Inc. (Madison, Wis.), some examples of which are described in U.S. Pat. Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand-held device, propelling the particles into a target tissue of interest. Other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, Oreg.), some examples of which are described in U.S. Pat. Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

[0195] Alternatively, micro-cannula- and microneedle-based devices (such as those being developed by Becton Dickinson and others) can be used to administer nucleic acid constructs of the invention. Illustrative devices of this type are described in EP 1 092 444 A1, and U.S. application Ser. No. 606,909, filed Jun. 29, 2000. Standard steel cannula can also be used for intra-dermal delivery using devices and methods as described in U.S. Ser. No. 417,671, filed Oct. 14, 1999. These methods and devices include the delivery of substances through narrow gauge (about 30 G) "micro-cannula" with limited depth of penetration, as defined by the total length of the cannula or the total length of the cannula that is exposed beyond a depth-limiting feature. It is within the scope of the present invention that targeted delivery of substances including nucleic acid

constructs can be achieved either through a single microcannula or an array of microcannula (or "microneedles"), for example 3-6 microneedles mounted on an injection device that may include or be attached to a reservoir in which the substance to be administered is contained.

2.4 Ancillary components

[0196] In some embodiments the composition further comprises one or more cytokines, which are suitably selected from flt3, SCF, IL-3, IL-6, GM-CSF, G-CSF, TNF-α, TNF-β, LT-β, IL-2, IL-7, IL-9, IL-15, IL-5, IL-1α, IL-1β, IFN-γ, IL-17, IL-16, IL-18, HGF, IL-11, MSP, FasL, TRAIL, TRANCE, LIGHT, TWEAK, CD27L, CD30L, CD40L, APRIL, TALL-1, 4-1BBL, OX40L, GITRL, IGF-I, IGF-II, HGF, MSP, FGF-a, FGF-b, FGF-3-19, NGF, BDNF, NTs, Tpo, Epo, Ang1-4, PDGF-AA, PDGF-BB, VEGF-A, VEGF-B, VEGF-C, VEGF-D, PIGF, EGF, TGF-α, AR, BTC, HRGs, HB-EGF, SMDF, OB, CT-1, CNTF, OSM, SCF, Flt-3L, M-CSF, MK and PTN or their functional, recombinant or chemical equivalents or homologues thereof. Preferably the cytokine is selected from the group consisting of IL-12, IL-3, IL-5, TNF, GMCSF, and IFN-γ.

3. Cell based therapy or prophylaxis

[0197] In accordance with the present invention, an inhibitor of IL-25 function, as described for example in Section 2.1, can be administered to a patient, together with antigen-presenting cells as described in Section 2.3.2 for priming or boosting an immune response. These cell based compositions are useful, therefore, for treating or preventing a disease or condition that is associated with the presence or aberrant expression of a target antigen. The cells of the invention can be introduced into a patient by any means (*e.g.*, injection), which produces the desired immune response to an antigen or group of antigens. The cells may be derived from the patient (*i.e.*, autologous cells) or from an individual or individuals who are MHC matched or mismatched (*i.e.*, allogeneic) with the patient. Typically, autologous cells are injected back into the patient from whom the source cells were obtained. The injection site may be mucosal, subcutaneous, intraperitoneal, intramuscular, intradermal, or intravenous. The cells may be administered to a patient to provide protective immunity or to a patient already suffering from a disease or condition or who is predisposed to a disease or condition in sufficient number to treat or prevent or alleviate the symptoms of the disease or condition. The number of cells injected into the patient in need of the treatment or prophylaxis may vary depending on *inter alia*, the antigen or antigens and size of the individual. This number may range for example between about 10^3 and 10^{11}, and usually between about 10^5 and 10^7 cells (*e.g.*, dendritic cells or T lymphocytes). Single or multiple administrations of the cells can be carried out with cell numbers and pattern being selected by the treating physician. The cells should be administered in a pharmaceutically acceptable carrier, which is non-toxic to the cells and the individual. Such carrier may be the growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. The cells may be administered alone or as an adjunct therapy in conjunction with other therapeutics known in the art for the treatment or prevention of unwanted immune responses for example but not limited to glucocorticoids, methotrexate, D-penicillamine, hydroxychloroquine, gold salts, sulfasalazine, TNFα or interleukin-1 inhibitors, and/or other forms of specific immunotherapy.

4. Preparation of immunostimulatory compositions

[0198] The preparation of the immunomodulating compositions of the present invention uses routine methods known to persons skilled in the art. Typically, such formulations and vaccines

are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredients are often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, phosphate buffered saline, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants that enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: surface active substances such as hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium bromide, N, N-dicoctadecyl-N', N'bis(2-hydroxyethyl-propanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as pyran, dextransulfate, poly IC carbopol; mineral gels such as aluminum phosphate, aluminum hydroxide or alum; peptides such as muramyl dipeptide and derivatives such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thur-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 1983A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, dimethylglycine, tuftsin; oil emulsions; trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion; lymphokines; QuilA and immune stimulating complexes (ISCOMS). For example, the effectiveness of an adjuvant may be determined by measuring antibody titres resulting from the administration of the immunostimulatory composition, wherein those antibodies are directed against one or more antigens presented by the treated cells of the immunostimulatory composition.

[0199] The active ingredients should be administered in a pharmaceutically acceptable carrier, which is non-toxic to the cells and the individual to be treated. Such carrier may be the growth medium in which the cells were grown. Compatible excipients include isotonic saline, with or without a physiologically compatible buffer like phosphate or HEPES and nutrients such as dextrose, physiologically compatible ions, or amino acids, and various culture media suitable for use with cell populations, particularly those devoid of other immunogenic components. Carrying reagents, such as albumin and blood plasma fractions and non-active thickening agents, may also be used. Non-active biological components, to the extent that they are present in the vaccine, are preferably derived from a syngeneic animal or human as that to be treated, and are even more preferably obtained previously from the subject. The injection site may be subcutaneous, intraperitoneal, intramuscular, intradermal, or intravenous. In preferred embodiments, the immunostimulatory composition is administered systemically. Preferably, the immunostimulatory composition is administered intramuscularly (*e.g.*, by intramuscular injection). In some embodiments, the immunostimulatory composition is administered by a route other than intranasally. Suitably, the immunostimulatory composition is administered by a route other than mucosally.

[0200] If soluble actives are employed (*e.g., a* soluble inhibitor of IL-25 function) active ingredients can be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free

carboxyl groups may also be derived from inorganic basis such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic basis as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0201] If desired, devices or pharmaceutical compositions or compositions containing the vaccine and suitable for sustained or intermittent release could be, in effect, implanted in the body or topically applied thereto for the relatively slow release of such materials into the body.

[0202] Techniques for formulation and administration may be found in "*Remington's Pharmaceutical Sciences*," Mack Publishing Co., Easton, Pa., latest edition. Suitable routes may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

[0203] The dosage to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. The dosage will also take into consideration the binding affinity of the inhibitor of IL-25 function to its target molecule, the immunogenicity of the immune stimulator, their bioavailability and their *in vivo* and pharmacokinetic properties. In this regard, precise amounts of the agent(s) for administration can also depend on the judgment of the practitioner. In determining the effective amount of the agent(s) to be administered in the treatment of a disease or condition, the physician or veterinarian may evaluate the progression of the disease or condition over time. In any event, those of skill in the art may readily determine suitable dosages of the agents of the invention without undue experimentation. Cell-containing compositions and vaccines are suitably administered to a patient in the range of between about 10^4 and 10^{10}, and more preferably between about 10^6 and 10^8 treated cells/administration. The dosage of the actives administered to a patient should be sufficient to effect a beneficial response in the patient over time such as a reduction in the symptoms associated with the cancer or tumor. For example, usual patient dosages for systemic administration of inhibitors of IL-25 function range from about 0.1-200 g/day, typically from about 1-160 g/day and more typically from about 10-70 g/day. Stated in terms of patient body weight, usual dosages range from about 1.5-3000 mg/kg/day, typically from about 15-2500 mg/kg/day, more typically from about 150-1000 mg/kg/day and even more typically from about 20-50 mg/kg/day.

[0204] Thus, the inhibitor of IL-25 function and the immune stimulator may be provided in effective amounts to stimulate or enhance the immune response to a target antigen.

5. Methods for stimulating immune responses

[0205] The compositions of the invention may be used for stimulating an immune response to a target antigen in a subject that is immunologically naïve to the target antigen or that has previously raised an immune response to that antigen. Thus, the present invention also extends to methods for enhancing an immune response in a subject by administering to the subject the compositions or vaccines of the invention. Advantageously, the immune response is a cell-mediated immune response (*e.g.*, a T-cell mediated response, which desirably includes $CD8^+$ IFN-γ-producing T cells).

[0206] Also encapsulated by the present invention is a method for treatment and/or prophylaxis of a disease or condition, comprising administering to a patient in need of such

treatment an effective amount of a inhibitor of IL-25 function, together with an effective amount of an immune stimulator, as broadly described above. In certain embodiments, the target antigen is associated with or responsible for a disease or condition which is suitably selected from cancers, infectious diseases and diseases characterized by immunodeficiency. Examples of cancer include but are not limited to ABL1 proto-oncogene, AIDS related cancers (*e.g.*, Kaposi's sarcoma), acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anemia, astrocytoma, ataxia telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, dermatofibrosarcoma protuberans, desmoplastic small round cell tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal carcinoid tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic disease, glioma, gynecological cancers, hematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhans' cell histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid tumor of kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small cell lung cancer (NSCLC), ocular cancers, esophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (SCLC), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia, Wilms' tumor.

[0207] In other embodiments, the composition of the invention could also be used for generating large numbers of $CD8^+$ or $CD4^+$ CTL, for adoptive transfer to immunodeficient individuals who are unable to mount normal immune responses. For example, antigen-specific $CD8^+$ CTL can be adoptively transferred for therapeutic purposes in individuals afflicted with HIV infection (Koup *et al.*, 1991, *J. Exp. Med.* 174: 1593-1600; Carmichael *et al.*, 1993, *J. Exp. Med.*

177: 249-256; and Johnson *et al.*, 1992, *J. Exp. Med.* 175: 961-971), malaria (Hill *et al.*, 1992, *Nature* 360: 434-439) and malignant tumors such as melanoma (Van der Brogen *et al.*, 1991, *Science* 254: 1643-1647; and Young and Steinman 1990, *J. Exp. Med.*, 171: 1315-1332).

[0208] In still other embodiments, the composition is suitable for treatment or prophylaxis of a viral, bacterial or parasitic infection. Viral infections contemplated by the present invention include, but are not restricted to, infections caused by HIV, Hepatitis, Influenza, Japanese encephalitis virus, Epstein-Barr virus and respiratory syncytial virus. Bacterial infections include, but are not restricted to, those caused by *Neisseria* species, *Meningococcal* species, *Haemophilus* species *Salmonella* species, *Streptococcal* species, *Legionella* species and *Mycobacterium* species (*e.g.*, *M. tuberculosis*). Parasitic infections encompassed by the invention include, but are not restricted to, those caused by *Plasmodium* species, *Schistosoma* species, *Leishmania* species, *Trypanosoma* species, *Toxoplasma* species and *Giardia* species.

[0209] The effectiveness of the immunization may be assessed using any suitable technique. For example, CTL lysis assays may be employed using stimulated splenocytes or peripheral blood mononuclear cells (PBMC) on peptide coated or recombinant virus infected cells using ^{51}Cr or Alamar Blue™ labeled target cells. Such assays can be performed using for example primate, mouse or human cells (Allen *et al.*, *J Immunol*, 2000, 164(9): 4968-4978 also Woodberry *et al.*, *infra*). Alternatively, the efficacy of the immunization may be monitored using one or more techniques including, but not limited to, HLA class I tetramer staining - of both fresh and stimulated PBMCs (see for example Allen *et al.*, *supra*), proliferation assays (Allen *et al.*, *supra*), ELISPOT assays and intracellular IFN-γ staining (Allen *et al.*, *supra*), ELISA Assays - for linear B cell responses; and Western blots of cell sample expressing the synthetic polynucleotides.

[0210] In some embodiments, the composition comprises a nucleic acid construct from which an antigen that corresponds to the target antigen is expressible. Administration of such nucleic acid constructs to a mammal, especially a human, may include delivery *via* direct oral intake, systemic injection, or delivery to selected tissue(s) or cells. Delivery of the nucleic acid constructs to cells or tissues of the mammal may be facilitated by microprojectile bombardment, liposome mediated transfection (*e.g.*, Lipofectin or Lipofectamine), electroporation, calcium phosphate or DEAE-dextran-mediated transfection, for example. A discussion of suitable delivery methods may be found in Chapter 9 of Ausubel *et al.*, (1994-1998, *supra*).

[0211] The step of introducing the expression vector into the selected target cell or tissue will differ depending on the intended use and species, and can involve one or more of non-viral and viral vectors, cationic liposomes, retroviruses, and adenoviruses such as, for example, described in Mulligan, R.C., (1993). Such methods can include, for example:

[0212] A. Local application of the expression vector by injection (Wolff *et al.*, *Science*, 1990, 247 (4949 Pt 1): 1465-1468), surgical implantation, instillation or any other means. This method can also be used in combination with local application by injection, surgical implantation, instillation or any other means, of cells responsive to the protein encoded by the expression vector so as to increase the effectiveness of that treatment. This method can also be used in combination with local application by injection, surgical implantation, instillation or any other means, of another factor or factors required for the activity of the protein.

[0213] B. General systemic delivery by injection of DNA, (Calabretta *et al.*, 1993), or RNA, alone or in combination with liposomes (Zhu *et al.*, 1993), viral capsids or nanoparticles (Bertling *et al.*, 1991) or any other mediator of delivery. Improved targeting might be achieved by linking the polynucleotide/expression vector to a targeting molecule (the so-called "magic bullet" approach employing, for example, an antigen-binding molecule), or by local application by injection, surgical implantation or any other means, of another factor or factors required for the activity of the protein encoded by the expression vector, or of cells responsive to the protein. For example, in the case of a liposome containing antisense IL-25 polynucleotides, the liposome may be targeted to skin cancer cells, *e.g.*, squamous carcinoma cells, by the incorporation of immuno-interactive agents into the liposome coat which are specific the EGF receptor, which is expressed at higher levels in skin cancer.

[0214] C. Injection or implantation or delivery by any means, of cells that have been modified *ex vivo* by transfection (for example, in the presence of calcium phosphate: Chen *et al.*, 1987, or of cationic lipids and polyamines: Rose *et al.*, 1991), infection, injection, electroporation (Shigekawa *et al.*, 1988) or any other way so as to increase the expression of the polynucleotide in those cells. The modification can be mediated by plasmid, bacteriophage, cosmid, viral (such as adenoviral or retroviral; Mulligan, 1993; Miller, 1992; Salmons *et al.*, 1993) or other vectors, or other agents of modification such as liposomes (Zhu *et al.*, 1993), viral capsids or nanoparticles (Bertling *et al.*, 1991), or any other mediator of modification. The use of cells as a delivery vehicle for genes or gene products has been described by Barr *et al.*, 1991 and by Dhawan *et al.*, 1991. Treated cells can be delivered in combination with any nutrient, growth factor, matrix or other agent that will promote their survival in the treated subject.

5.1 Prime-boost regimens

[0215] The methods of the invention may comprise administering a priming composition of an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject, and subsequently administering a later booster composition of the target antigen together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible as broadly defined above and elsewhere herein.

[0216] For example, the booster composition may be administered at least 7, 14, 21 or 28 days, at least 1, 2, 3, 4, 5, or 6 months, or at least 1, 2, 3, 4, or 5 years after the priming composition. The immune stimulator and the inhibitor of IL-25 function may be administered separately or sequentially. The priming and booster compositions may be administered by the same or different routes. For example, the priming and booster doses may both be administered - mucosally (*e.g.*, intranasally), intramuscularly, intravenously, or subcutaneously. Alternatively, the priming dose may be administered locally (*e.g.*, mucosally, such as intranasally) to induce mucosal antigen-specific immune cells, and the booster dose administered intramuscularly, subcutaneously, or intravenously to induce systemic antigen-specific immune cells. Preferably, the booster dose is administered intramuscularly. In preferred embodiments, the booster dose is administered by a route other than intranasally. Suitably, the booster dose is administered by a route other than mucosally.

[0217] Optionally, the priming dose of immune stimulator further comprises an inhibitor of IL-4 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-4 is expressible. The inhibitor of IL-4 function includes any molecule or compound that directly or indirectly binds or physically associates with IL-4 or its receptor(s) and that suitably blocks, inhibits or otherwise antagonizes at least one of its functions or activities (*e.g.*, binding to or interaction with one or more surface molecules (*e.g.*, receptors) present on white blood cells, especially lymphocytes and more especially T lymphocytes). The binding or association may involve the formation of an induced magnetic field or paramagnetic field, covalent bond formation, an ionic interaction such as, for example, occur in an ionic lattice, a hydrogen bond or alternatively, a van der Waals interactions such as, for example, a dipole-dipole interaction, dipole-induced-dipole interaction, induced-dipole-induced-dipole interaction or a repulsive interaction or any combination of the above forces of attraction.

[0218] In certain embodiments, the inhibitor of IL-4 function is any molecule capable of specifically preventing activation of cellular receptors for IL-4. For example, inhibitors of this type can be selected from soluble native or variant IL-4 receptors or soluble IL-4 receptor subunits.

[0219] In certain embodiments, the inhibitor of IL-4 function is a variant form of IL-4, including but not limited to IL-4C123 or AEROVANT™ (AER 001, pitrakinra produced by Aerovance) which is a 15 kDa recombinant human IL-4 mutein (*see*, *The Lancet*, 2007, 370: 1422-1431).

[0220] Alternatively, such an inhibitor can be an antigen-binding molecule that is immuno-interactive with an IL-4 receptor. In these embodiments, the antigen-binding molecule may bind to the IL-4 receptor but will not signal via the receptor, thus blocking any host IL-4 signaling. In other embodiments, the inhibitor of IL-4 function is an antigen-binding molecule that is immuno-interactive with at least a portion of IL-4. In these embodiments, the antigen-binding molecules can be immuno-interactive with an active or an inactive form of IL-4, the difference being that antigen-binding molecules to the active cytokine are more likely to recognise epitopes that are only present in the active conformation. Representative examples of such inhibitors include ligands or single-chain antibodies.

[0221] In some embodiments, the second agent and the third agent may comprise the same molecule. Notably, in specific embodiments, the second agent and the third agent comprises the IL-4 variant, IL-4C123, which can bind to both the IL-4 type 1 receptor and the IL-4 type 2 receptor preventing cellular signalling through these pathways. An exemplary IL-4C123 polypeptide sequence is set out in SEQ ID NO: 100 (suitably encoded by the optimized nucleic acid sequence set forth in SEQ ID NO: 101),

wherein:

SEQ ID NO: 100 is:
HKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAASKNTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQQ
FHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKTIMREK; and

SEQ ID NO: 101 is:
ATGGGCCTGACCAGCCAGCTGCTGCCCCCCCTGTTCTTCCTGCTGGCCTGCGCCGGCAACTTCGTGC
ACGGCCACAAGTGCGACATCACCCTGCAGGAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAA
GACCCTGTGCACCGAGCTGACCGTGACCGACATCTTCGCCGCCAGCAAGAACACCACCGAGAAGGA
GACCTTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCACGAGAAGGACACCAGATGC

CTGGGCGCCACCGCCCAGCAGTTCCACAGACACAAGCAGCTGATCAGATTCCTGAAGAGACTGGACA GAAACCTGTGGGGCCTGGCCGGCCTGAACAGCTGCCCCGTGAAGGAGGCCAACCAGAGCACCCTGG AGAACTTCCTGGAGAGACTGAAGACCATCATGAGAGAGAAGTGA.

6. Kits

[0222] The present invention also provides kits comprising an immunostimulatory compositions as broadly described above and elsewhere herein. Such kits may additionally comprises alternative immunogenic agents for concurrent use with the immunostimulatory compositions of the invention.

[0223] In some embodiments, in addition to the immunostimulatory compositions of the present invention the kit may include suitable components for performing the prime-boost regiments described above. For example, the kit may include a priming dose of the immune stimulator. Furthermore, the priming dose may also comprise an inhibitor if IL-4 function, as described in detail, above.

[0224] The kit may comprise additional components to assist in performing the methods of the present invention such as, for example, administration device(s), buffer(s), and/or diluent(s). The kits may include containers for housing the various components and instructions for using the kit components in the methods of the present invention.

[0225] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

ENHANCING IMMUNITY THROUGH INHIBITION OF IL-25 FUNCTION

Materials & Methods

Mice

[0226] 5-8-week-old pathogen free female BALB/c mice were obtained from the Australian Phenomics Facility, the Australian National University. Mice were handled and maintained under the Australian National University Animal Experimentation and Ethics Committee approved guidelines, protocol numbers A2014/14 and A2017-15.

Vaccine Preparation

IL-25BP gene and isolation of recombinant poxviruses

[0227] The synthetic gene for secreted IL-25BP encodes the signal peptide (removed during post-translational processing) and extracellular binding domains of mouse IL-17RB, the transmembrane and cytoplasmic domains are not included. IL-17RB is known to bind IL-17B (poorly defined immune activity) and IL-25 (also known as IL-17E). The synthetic IL-25BP gene was further codon optimized for efficient expression in mouse cells. The addition of an upstream in-frame coding sequence and BamHI restriction site allows in-frame ligation into the Fowlpox vector pAF09 early-late (TAAATG) promoter (*see*, Heine, H.G., Boyle, D.B., *Arch Virol*, 1993, 131(3-4):

277-92). A downstream HindIII restriction site was included to facilitate sub-cloning. The following synthetic DNA sequence was custom prepared by GenScript USA/HK:

[0228] ATGCTGCTGGTGCTGCTGATCCTGGCCGCCAGCTGCAGGAGCGCCCTGCCTAGGGAG
CCTACCATCCAGTGCGGCAGCGAGACCGGCCCTAGCCCTGAGTGGATGGTGCAGCACACCCTGACCCCTGG
CGACCTGAGGGACCTGCAGGTGGAGCTGGTGAAGACCAGCGTGGCCGCCGAGGAGTTCAGCATCCTGATG
AACATCAGCATCCTGAGGGCCGACGCCAGCATCAGGCTGCTGAAGGCCACCAAGATCTGCGTGAGCGGCAA
GAACAACATGAACAGCTACAGCTGCGTGAGGTGCAACTACACCGAGGCCTTCCAGAGCCAGACCAGGCCTA
GCGGCGGCAAGTGGACCTTCAGCTACGTGGGCTTCCCTGTGGAGCTGAGCACCCTGTACCTGATCAGCGCC
CACAACATCCCTAACGCCAACATGAACGAGGACAGCCCTAGCCTGAGCGTGAACTTCACCAGCCCTGGCTGC
CTGAACCACGTGATGAAGTACAAGAAGCAGTGCACCGAGGCCGGCAGCCTGTGGGACCCTGACATCACCGC
CTGCAAGAAGAACGAGAAGATGGTGGAGGTGAACTTCACCACCAACCCTCTGGGCAACAGGTACACCATCC
TGATCCAGAGGGACACCACCCTGGGCTTCAGCAGGGTGCTGGAGAACAAGCTGATGAGGACCAGCGTGGC
CATCCCTGTGACCGAGGAGAGCGAGGGCGCCGTGGTGCAGCTGACCCCTTACCTGCACACCTGCGGCAACG
ACTGCATCAGGAGGGAGGGCACCGTGGTGCTGTGCAGCGAGACCAGCGCCCCTATCCCTCCTGACGACAAC
AGGAGGATGCTGGGCGGCTGA [SEQ ID NO: 102]

Recombinant fowlpox virus

[0229] The synthetic IL-25BP DNA was ligated between the BamHI and HindIII sites of the FPV vector pAF09 (*see*, Heine, *supra*) (Figure 1A). The IL-25BP gene is indicated by a solid red arrow downstream of the FPV early-late promoter. The flanking FPV genes are shown as black arrows. Selectable markers xanthine guanine phosphoribosyl transferase (ECOGPT, green) and β-galactosidase (BGAL LacZ, blue) are shown.

[0230] Recombinant fowlpox viruses co-expressing HIVgag and IL-25BP was constructed using parent virus FPV-HIV (HIVgagpol/env AE) (*see*, Coupar, B.E., *et al.*, *Vaccine*, 2006, 24(9): 1378-88). The IL-25BP gene was transferred into the FPV-HIV genome by homologous recombination using Lipofectamine 2000 mediated plasmid DNA transfection of FPV-HIV infected chick embryo skin cells, mycophenolic acid/xanthine selection and identification of β-galactosidase positive recombinant plaques using X-gal staining (*see*, Heine, *supra*; and Jackson, R.J., Boyle, D.B., Ranasinghe, C., *Methods Mol Biol*, 2014, 1143: 61-90).

Recombinant MVA virus

[0231] The IL-25BP gene as ligated between the BamHI and HindIII sites of the MVA vector pMVAdel III-TDS (Ronald Jackson, *unpublished*) downstream of a early/late promoter, including a HindIII cassette containing the Psyn-β-glucuronidase (GUS) gene (Figure 1B). This transfer vector contains an insertion site located within the natural deletion-III region of MVA between open reading frames MVA164 and MVA165. Recombinant MVA expressing IL-25BP was isolated by Lipofectamine 2000 mediated plasmid transfection of MVA infected chick embryo skin cells, using the transient dominant selection procedure (*see*, Falkner, F.G., Moss, B., *J Virol*, 1990, 64(6): 3108-11) of the green fluorescent protein expression and Blasticidin S resistance and identification of recombinant virus plaques expressing GUS using X-gluc staining (*see*, Jackson, R.J., Hall, D.F., Kerr, P.J., *J Gen Virol*, 1996, 77(7): 1569-75).

Viruses

[0232] FPV-HIV expresses HIV genes gag-pol and env genes (AE clade) inserted into the "F" and "REV" sites respectively, was described by Coupar *et al*. FPV has abortive replication in mammals, however expresses both early and late promoter controlled viral genes.

[0233] MVA is the attenuated Modified Vaccinia Ankara vaccine strain of vaccinia virus that has abortive replication in mammals (*see*, Kennedy, J.S., Greenberg, R.N., *Expert Rev Vaccines*, 2009, 8(1), 13-24) and expresses both early and late promoter controlled viral genes.

[0234] MVA-HIVgagpol(AE) was previously constructed as follows: polymerase chain reaction isolation of the fowlpox virus early/late promoter and HIVgag-pol genes from FPV092 and sub-cloning into the MVA vector MVA-F-GFPBSD (Ronald Jackson, *unpublished*) into a synthetic intergenic region between MVA036(F7L) and MVA037(F8L) open reading frames. Recombinant MVA-HIVgagpol(AE) was isolated as Green Fluorescent Protein positive, Blasticidin S resistant plaques (Ronald Jackson, *unpublished*).

Immunization of Mice

ILC immunization studies

[0235] BALB/c mice were administered 1 x 10^7 plaque forming units (PFU) rFPV control or rFPV-IL-25BP vaccines (expressing the HIV or TB antigens) by intramuscular (i.m.) injection. The i.m. vaccines were administered as 50 µl quadriceps muscle. All vaccines were diluted in sterile phosphate buffered saline (PBS) and sonicated three times for 15 seconds at 50% output using a Branson Sonifier 450 to distribute virus particles prior to use.

Prime-boost vaccination

[0236] Prime immunization FPV-HIV (1x10^7 PFU) was administered intra-nasally as described previously (*see*, Ranasinghe 2013; and Jackson 2014a, *supra*) Two weeks following the i.n. prime immunization a mixture of MVA-HIV (1x10^7 PFU) and MVA-IL25BP (1x10^7 PFU) was administered by i.m. injection to boost the mucosal and systemic HIV or TB specific immune responses. This was compared to the control prime-boost vaccination using the non-adjuvant i.n. FPV-HIV / i.m. MVA-HIV. Two weeks post boost immunization $HIVgag_{(197-205)}$ specific $CD8^+$ T cell responses in the Peyer's patches (mucosal immunity) and spleen (systemic immunity) were analyzed for cytokine poly-functionality by intracellular cytokine staining for IFN-γ, TNF-α, IL-17A and IL-22 using methods described previously (*see*, Jackson 2014a; Ranasinghe, 2013; Jackson 2014b, *supra*; and Ravichandran, J., *et al*., *Cytokine Res*, 2015, 35(3): 176-85).

Preparation of Single Cell Suspensions

[0237] For Innate Lymphoid Cell (ILC) studies, muscle cells were isolated 24 hours post immunization. Vaccinated muscle was cut into < 5 mm pieces and digested with 0.5 mg/ml collagenase, 2.4 mg/ml dispase, 5 Units/ml DNase in complete RPMI for 30 minutes at 37°C, then passed through a Falcon cell strainer to remove debris. The separated cells were washed and suspended in complete RPMI (5% FBS), rested overnight at 37°C with 5% to allow recovery of cell surface markers and then stained for multi-color flow cytometry analysis.

[0238] For $CD8^+$ T cell analysis, mice were euthanized at two weeks post-boost immunization. Spleen and intestinal Peyer's patches were removed and single cell suspensions

prepared in complete (5% FBS) RPMI. Spleens were dissociated through a Falcon cell strainer and suspended in red blood cell lysis buffer (0.15 mM NH_4Cl, 10.0 mM $KHCO_3$, 0.1 mM Na_2EDTA) at room temperature for 10 minutes to lyse the red blood cells. The cells were then washed and suspended in complete RPMI. Similarly, Peyer's patch mucosal lymphocytes were prepared as for spleen without the red cell lysis step (as described in Jackson, 2014a).

Flow Cytometry

[0239] Monoclonal antibodies FITC-conjugated anti-mouse CD3 (clone 17A2), CD19 (clone 6D5), CD11b (clone M1/70), CD11c (clone N418), CD49 (clone HMα2), FcεRIα (clone MAR-1) (all linage positive markers were selected as FITC). Linage positive cells are: $CD3^+$ T cells, $CD19^+$ B cells, $CD11c^+$ $CD11b^+$ macrophages and dendritic cells, $CD49^+$ NK cells, $FcεRIα^+$ mast cells and basophils. Lineage negative cells do not express the recognised cell surface markers of lineage-positive immune cells and contain the recently identified Innate Lymphoid Cell (ILC) populations.

[0240] PE-conjugated anti-mouse ST2/IL-33R (clone DIH9), APC-conjugated anti-mouse IL-17RB (IL-25R, clone 9B10), APC/Cy7-conjugated anti-mouse CD45 (clone 30-F11), Brilliant Violet 421-conjugated anti-mouse CD335 (NKp46) (clone 29A1.4), Brilliant Violet 421-conjugated anti-mouse IL-4 (clone 11B11), Brilliant Violet 510-conjugated anti-mouse IFN-γ (clone XMG1.2), APC-conjugated IL-22 (clone Poly5164), Alexa Fluor 700-conjugated IL-17A (clone TC11-18H10.1) were obtained from BIOLEGEND. PE-eFlour 610-conjugated anti-mouse IL-13 (clone eBio13A) was purchased from eBioscience.

[0241] The ILC cell surface and intracellular cytokine staining were performed according established protocols (*see*, Jackson 2014a; Ranasinghe, 2013; Jackson 2014b, and Ravichandran, 2015 *supra*), fixed with 0.5% paraformaldehyde, and run on a BD LSR Fortessa. From each sample 2 x 10^6 events were acquired and data were analyzed with Tree Star FlowJo software (version 10.0.7 for Windows) using a gating strategy adapted from Kim *et al.*, 2012 (Kim, H.Y., *et al.*, *J Allergy Clin*, 2012, 129(1): 216-27).

[0242] APC-conjugated $K_dGag_{197-205}$ tetramers were synthesized at the Bio-Molecular Resource Facility at The John Curtin School of Medical Research, ANU. Splenocytes or mucosal lymphocytes (2 - 5 x 10^6) were stained with anti-CD8-FITC antibody (BIOLEGEND) and APC-conjugated $K_dGag_{197-205}$ tetramer and analyzed by intracellular cytokine staining and flow cytometry as previously described (*see*, Jackson 2014a; Ranasinghe, 2013; Jackson 2014b, and Ravichandran, 2015 *supra*).

Results

Analysis of ILC in Response to Intramuscular Immunization with FPV Vaccine

ILC2 cells

[0243] Mice were given intramuscular immunizations with non-adjuvant FPV vaccine. Innate Lymphoid cells (ILC) present in the inoculated muscle were prepared 24 hours post immunization, then analyzed by flow cytometry (Figure 2). $CD45^+$, Lineage-negative (Lin^-) cells were further analyzed for surface ST2 (IL-33R). Data demonstrate that muscle ILC2 do not express surface IL-33R, but do express IL-25R (IL-17RB). When the muscle $IL\text{-}25R^+$ ILC2 were further

characterized for IL-13 and IL-4 expression, data revealed that activated muscle ILC2 express IL-13, but not IL-4.

ILC1 & ILC3 cells

[0244] Muscle $CD45^+$, Lin^-, $IL\text{-}33R^-$ were further analyzed for surface $NKp46^+$ and $NKp46^-$ populations (Figure 3). The $NKp46^+$ and $NKp46^-$ populations were further characterized for expression of interferon-γ (ILC1) or IL-17A and IL-22 (ILC3) expressing cells (Figure 4). The flow cytometry data indicates that Lin^- $NKp46^+$ cells express IFN-γ, IL-17A and IL-22 and therefore contain both ILC1 and ILC3 populations. Notably, the Lin^- $NKp46^-$ cells express IL-17A and IL-22 (and not IFN-γ), indicating the presence of only ILC3 populations.

Analysis of ILC in Response to Intramuscular Immunization with FPV-IL25BP Vaccine

ILC2 cells

[0245] BALB/c mice were immunized by intramuscular FPV-IL25BP vaccination and ILC2 ($CD45^+$, Lin^-, $ST2^-$, $IL\text{-}25R^+$) populations analyzed 24 hours post-immunization (Figure 5). Data indicate that in response to FPV-IL25BP vaccination, muscle ILC2 do not express IL-33R. All Lin^- ILC that were induced (99%) were $IL\text{-}25R^+$, in comparison to the non-adjuvant control FPV-HIV vaccine strategy where only ~ 6% of the ILC were $IL\text{-}25R^+$ ILC2 (Figure 2). Intracellular cytokine staining indicated these $IL\text{-}25R^+$ ILC2 did not express IL-13 or IL-4. This data indicates that transient inhibition of IL-25R signaling, by sequestering free IL-25 in muscle by FPV-IL25BP vaccination, completely inhibited activation and IL-13 production by $CD45^+$ Lin^- $IL\text{-}33R^-$ $IL\text{-}25R^+$ ILC2 compared to non-adjuvanted i.m. administration of FPV-HIV vaccination (Figure 2).

ILC1 & ILC3 cells

[0246] BALB/c mice were immunized by i.m. FPV-IL25BP vaccination and ILC1-like and ILC3-like cells evaluated 24 hours post vaccination (Figure 6). Data indicates that following FPV-IL25BP vaccination the majority of $CD45^+$ Lin^- $IL\text{-}33R/ST2^-$ ILC3-like cells were $NKp46^-$ (99%) and express elevated IL-17A (~ 98%) and IL-22 (~ 98%), but no or little IFN-γ expression was detected by "ILC1-like" cells. Furthermore, no appreciable $NKp46^+$ populations (< 1.5%) were detected compared with non-adjuvanted i.m. FPV-HIV vaccination (~ 86%) (*see*, Figures 3 & 4).

Prime-Boost Immunization with FPV-HIVgag/MVA-HIVgag-IL25BP Vaccines

[0247] Twenty-four hours post i.n. FPV-HIV vaccination, lung mucosal ILC2 are exclusively $IL\text{-}33R^+$ ($ST2^+$) and express IL-13. In contrast, 24 hours post i.m. vaccination FPV-HIV vaccination muscle ILC2 cells are exclusively $IL\text{-}25R^+$ and express IL-13. Intranasal vaccination with FPV-HIV also induces IL-22 expression, however, it does not induce IL-17A expression by mucosal Lin^- $ST2^-$. In contrast, i.m. FPV-HIV immunization induces muscle ILC3 cells expressing both IL-22 and IL-17A. These results indicate that lung ILC2 and muscle ILC2 are distinct populations with respect to cell receptor expression and cytokine repertoire.

[0248] The results presented herein show that transient inhibition of IL-25R signaling of muscle ILC2, by sequestering free IL-25 using a soluble IL-25BP expressed during vaccination, inhibits activation and maturation of $IL\text{-}25R^+$ ILC2. Nearly 100% of the Lin^- ILC cells differentiate to a $NKp46^-$ ILC3-like phenotype expressing both IL-17A and IL-22. Interestingly, these ILC3-like cells

are also IL-25R$^+$, having a mixed ILC2 (surface receptor) and ILC3 (cytokine) phenotype. (CD45$^+$ Lin$^-$ IL-33R$^-$ IL-25R$^+$ NKp46$^-$ IL-17A$^+$ IL-22$^+$).

[0249] The present inventors performed a heterologous recombinant poxvirus prime-boost vaccination directed at HIVgag antigen T cell immunity to determine whether transient inhibition of IL-25 signaling during the i.m. vaccination would enhance antigen-specific T cells. BALB/c mice (n = 4/group) were i.n. primed with recombinant FPV-HIV and then two weeks later i.m. boosted with either an equal mixture of MVA-HIV/MVA-IL25BP adjuvant vaccine, or a MVA-HIV vaccine control.

[0250] Following i.m. vaccine boosting, HIVgag specific CD8$^+$ T cells were measured by Kd-gag-specific tetramer staining of spleen (systemic) and Peyer's patch (mucosal) cells (Figures 7 and 8). Significantly elevated numbers of HIVgag specific CD8$^+$ cells were detected in Peyer's patch with the adjuvanted vaccine strategy compared to the control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV prime-boost vaccine strategy.

[0251] Spleen and Peyer's patch HIVgag specific CD8$^+$ T cells were evaluated for IFN-γ expression following prime-boost FPV-HIV/MVA-HIV-IL25BP vaccination (Figure 9 and 10). Elevated numbers of HIVgag specific CD8$^+$ cells expressing IFN-γ were detected in Peyer's patch relative to control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV vaccination strategy.

[0252] Spleen and Peyer's patch HIVgag specific CD8$^+$ T cells were evaluated for TNFα expression following prime boost FPV-HIV/MVA-HIV-IL25BP vaccination (Figure 11). Although no significant differences were detected in the mucosal compartment (Peyer's patch), elevated numbers of TNF-α expressing HIVgag specific CD8$^+$ cells were observed in spleen relative to the control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV vaccination strategy.

[0253] CD8$^+$ T cells were evaluated for polyfunctional (IFN-γ and TNF-α expression) following i.n. FPV-HIV/i.m. MVA-HIV-IL25BP prime-boost (Figure 12). Although no differences were detected in the systemic (spleen) compartment comparing the vaccine strategies, elevated numbers of polyfunctional HIVgag specific CD8$^+$ cells were detected in Peyer's patch relative to the control unadjuvanted i.n. FPV-HIV/ i.m. MVA-HIV vaccination strategy.

[0254] Transient inhibition of IL-25 signaling during muscle vaccination resulted in a highly significant enhancement of the IL-17A and IL-22 expression by ILC3-like cells.

[0255] Evaluation of IL-17A and IL-22 expressing HIVgag-specific systemic (spleen) CD8$^+$ T cells following an i.n. FPV-HIV prime vaccination and an i.m. MVA-HIV-IL25BP booster vaccination is shown (Figure 13). Elevated numbers of HIVgag specific CD8$^+$ T cells expressing IL-22 and IL-17A were detected in spleen relative to the control unadjuvanted vaccine strategy. However, this elevation was low compared to IFN-γ or TNF-α expression.

[0256] Figure 14 provides a clear evaluation of IL-17A and IL-22 expressing HIVgag-specific CD8$^+$ T cells in Peyer's patch following FPV-HIV i.n. prime and i.m. MVA-HIV-IL25BP boost vaccination. Although low compared to IFN-γ and TNF-α expression, elevated numbers of HIVgag-specific CD8$^+$ T cells expressed IL-22 and IL-17A were detected in Peyer's patches relative to the control unadjuvanted vaccination.

[0257] In summary, providing a soluble inhibitor for IL-25 during the intramuscular boost vaccination resulted in elevated HIVgag-specific CD8$^+$ T cell response in both the systemic

and mucosal immune compartments, with enhanced numbers IFN-γ TNF-α, IL-22 and IL-17A expressing T cells, importantly in the mucosae where pathogens are first encountered. The ability to enhance both Th1 and Th17 mediated response at mucosal surfaces would be beneficial for many vaccines, particularly for immunity for pulmonary tuberculosis where it is recognised $CD4^+$ T cell expression of IFN-γ and IL-17A is required for effective immunity.

[0258] The IL-25BP i.m. boost could be also used in conjunction with an IL-4R antagonist. Providing the IL-4R antagonist in the i.n. prime vaccination should enhance Th1 mediated immunity with increased antigen-specific $CD8^+$ T avidity and poly-functional cytokine expression (IFN-γ, TNF-α, IL-2) (*see*, Jackson, 2014b, *supra*). An i.m. boost including an inhibitor of IL-25 function, such as IL-25BP adjuvant, could be used to enhance antigen specific IL-17A/IL-22 T cell responses, providing combined enhanced Th1 and Th17 mediated responses in both mucosal and systemic compartments.

[0259] These results indicate that IL-13 expressed by ILC2 negatively regulates Th1 mediated anti-viral $CD8^+$ T cell immunity, while also positively (possibly via an alternative IL-13 receptor) and negatively (*via* IL-4Rα/IL13Rα1 STAT6 signaling) influencing ILC1 IFN-γ expression and antibody immunity by an uncharacterized mechanism.

[0260] These studies indicate there are complex interactions between ILC and antigen presenting cells (APC, dendritic cells and macrophages) with the cytokines expressed by ILC influencing APC activation and development of adaptive $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, and B cells and antibody expression.

Discussion

[0261] It is proposed that a recombinant vaccine co-expressing an antigen and an IL-25 inhibitor (such as IL-25BP) will be able to elicit T cells with higher avidity to antigens and a capacity to protect against a pathogenic challenge. Thus, the co-expression of an antigen and a soluble IL-25 antagonist in a recombinant vaccine will be able to dramatically enhance T cell avidity elicited to vaccine antigens and afford a higher level of protection against challenge.

[0262] It is considered that the vector of the vaccine will enter cells of the host, some of which will be antigen-presenting cells of the immune system, which will express both the antigen and the IL-25 antagonist. The antigen is processed by the cell so as to stimulate an immune response specific for the antigen, while the IL-25 antagonist leaves the cell and binds to host IL-25 that is produced during the immune response. The expression of both the antigen and IL-25 antagonist occurs in the local milieu of the immune response. The present inventors propose that production of both the antigen and IL-25 antagonist in the local milieu of the immune response may be an essential requirement for the desired immune response (production of high avidity $CD8^+$ T cells). It is further proposed that the delivery of the antigen and IL-25 antagonist separately fails to induce appropriate responses. In the recombinant vaccine of the present invention, the IL-25 antagonist binds and therefore detracts host IL-25 from its negative effect on the immune response resulting in heightened T cells responses with increased avidity towards the antigen. The T cells elicited under this regime also have markedly broadened cytokine profile responses different from that induced without the IL-25 antagonist.

[0263] Accordingly, it is considered that such vaccines will be useful in providing improved immunity to the recipient as they inhibit the development of detrimental Th2 host responses.

EXAMPLE 2

PRIME-BOOST VACCINATION STUDY – EVALUATION OF $CD4^+$ AND $CD8^+$ T CELL IMMUNITY

[0264] WT BALB/c mice were prime-boost immunized two weeks apart using intranasal/ intramuscular (i.n./i.m.) vaccine strategy with; (1) rFPV $1x10^7$ PFU /rMVA $2x10^7$ PFU, (2) rFPV-IL-25BP $1x10^7$ PFU /rMVA-IL-25BP $2x10^7$ PFU and (3) rFPV-IL-4R antagonist $1x10^7$ PFU / rMVA-IL-25BP $2x10^7$ PFU, expressing TB antigens. These constructs were prepared using analogous protocols to those described in Example 1. $CD4^+$ and $CD8^+$ T cell immune responses were evaluated 14 days post booster immunization.

[0265] Data from this study revealed the following:

[0266] Similar to the HIV vaccine studies, although no differences in cytokine production by $CD4^+$ or $CD8^+$ T cells were observed in spleen (systemic compartment) following IL-25BP vaccination, significant differences were observed in lung and the mucosal compartment (Figure 15);

[0267] IL-25BP adjuvant vaccine given i.m. can manipulate the ILC at the vaccination site (muscle), and help recruit unique antigen-presenting cells that imprint T cells home to the mucosae. Accordingly, the present inventors propose that co-administration of a IL-25 inhibitor and an antigen that corresponds to a target antigen via a systemic route will enhance mucosal immunity to the target antigen.

[0268] Based on these results, the present inventors propose that i.m./i.m. delivery of IL-25R antagonist will generate long lasting mucosal immunity. This would be of great value given the safety associated with i.m. delivery compared to i.n. delivery.

[0269] IL-4R antagonist adjuvant prime followed by IL-25BP adjuvant booster induced highly multi-functional $CD4^+$ and $CD8^+$ T cells expressing IFN-γ, IL-2 and IL-17A compared to strategies 1 and 2 above. (production of these cytokines is a hallmark of protective efficacy against both HIV and TB).

[0270] Furthermore, following i.n. rFPV-L-4R antagonist/ i.m. rMVA- IL-25BP prime-boost strategy, the ability to produce IL-17A by $CD4^+$ and $CD8^+$ T cells appeared to be superior to i.n./i.m. IL-4R antagonist adjuvanted (alone) prime-boost vaccination strategy.

EXAMPLE 3

*INTRANASAL **DELIVERY OF IL-25BP** - ILC STUDY*

[0271] When rFPV co-expressing IL-25BP was given i.n. ($1x10^7$ PFU) to BALB/c mice, other than the lung resident IL-$33R^+$ ILC2, unexpected IL-$25R^+$ ILC2 and $TSLPR^+$ ILC2 populations were detected compared to unadjuvanted rFPV vaccination (see, Figure 16).

[0272] Unlike i.m. delivery, when IL-25BP was given i.n. although it inhibited IL-13 production by lung resident IL-$33R^+$ ILC2, it also unexpectedly enhanced IL-13 expression by IL-$25R^+$ ILC2 and also IL-4 and IL-13 expression by $TSLPR^+$ ILC2, and also a new ILC2 subset

(interestingly, the latter three types of ILC2 have not been detected in the lung following unadjuvanted rFPV vaccination) (Figures 16 and 17).

[0273] The new ILC2 subset (IL-33R$^-$ IL-25R$^-$ TSLPR$^-$ ILC2) was shown to induce elevated IL-4 and IL-13 compared to any of the other three LC2 subsets (Figure 18d far right) **.

[0274] Furthermore, IL-25BP adjuvanted rFPV vaccination was shown to enhance the NKP46+ ILC1 and 3 numbers (Figure 19), including their expression of IFN-γ and IL-17A (Figure 20) compared to unadjuvanted vaccination.

[0275] In conclusion, the results of this study indicate that delivery of IL-25BP should be performed suitably by a route other than a mucosal route (*e.g.*, other than i.n.) and preferably by a systemic route of administration (*e.g.*, i.m.) to block IL-25R$^+$ ILC2 expressing IL-13 in muscle but not i.n to manipulate lung ILC2.

[0276] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0277] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0278] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

CLAIMS

1. An immunostimulatory composition comprising a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject, together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible.

2. The composition of claim 1, wherein the composition comprises a nucleic acid composition comprising: a first agent comprising a coding sequence for an immune stimulator operably linked to a regulatory polynucleotide, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject; and a second agent comprising a coding sequence for an inhibitor of IL-25 function operably linked to a regulatory polynucleotide.

3. The composition of claim 1 or claim 2, wherein the first agent and the second agent are in the form of a single composition.

4. The composition of claim 2, wherein the first agent and the second agent are in the form of one or more nucleic acid constructs.

5. The composition of any one of claims 1 to 4, wherein the composition is formulated such that the first agent and the second agent are co-expressed in the subject.

6. The composition of any one of claims 1 to 5, wherein the composition is formulated for intramuscular injection.

7. The composition of any one of claims 1-6, wherein the target antigen is derived from a cancer or tumor, or a pathogenic organism.

8. The composition of claim 7, wherein the pathogenic organism is a virus, fungi, bacteria, parasite, algae, protozoa or amoebae.

9. The composition of claim 8, wherein the virus is selected from human immunodeficiency virus (HIV), hepatitis (A-E), influenzavirus A-D, human parainfluenza virus, measles virus, and rubella virus (RuV).

10. The composition of claim 9, wherein the HIV antigen is selected from any one of the gene product of the *gag*, *pol*, or *env* genes, the Nef protein, reverse transcriptase, and other HIV components.

11. The composition of claim 9, wherein the hepatitis antigen is selected from the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, *e.g.*, hepatitis A, B, and C, viral components such as hepatitis C viral RNA.

12. The composition of claim 9, wherein the influenzavirus antigen is selected from as hemagglutinin and neuraminidase, and other influenza viral components.

13. The composition of claim 8, wherein the bacteria is selected from *Mycobacterium tuberculosis* (TB), *M. bovis*, and *M. avium paratuberculosis*.

14. The composition of any one of claims 1-13 for use in therapy.

15. A kit comprising a priming composition comprising a first agent comprising an immune stimulator or a polynucleotide from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to a target antigen in a subject, and a boosting composition comprising the immune stimulator

together with a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible.

16. The kit of claim 15, wherein the priming composition further comprises an inhibitor of IL-4 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-4 function is expressible.

17. The kit of claim 16, wherein the priming composition is in the form of a single composition.

18. The kit of any one of claim 14 to 17, wherein the boosting composition is in the form of a single composition.

19. A method of eliciting an immune response to a target antigen in a subject, the method comprising concurrently administering to the subject the first agent and the second agent as defined in claim 1.

20. The method of claim 19, wherein the agents are administered systemically.

21. The method of claim 19 or claim 20, wherein the agents are administered intramuscularly.

22. The method of any one of claims 19 to 21, wherein the second agent contacts an ILC2 cell.

23. The method of any one of claims 19 to 22, wherein the immune response includes a Th17 immune response.

24. The method of claim 23, wherein the Th17 immune response comprises an increase in the expression of any one of IL-17A, IL-22, TNF-α, IL-17F, IL-21, IL-26, GM-CSF, and MIP-A.

25. The method of any one of claims 19 to 24, wherein the immune response includes a Th1 immune response.

26.

27. The method of claim 25, wherein the Th1 immune response comprises an increase in the expression of any one of IFN-γ, IL-2 and TNF-β.

28. The method of any one of claims 19 to 26, wherein the number of ILC3-like cells (*i.e.*, ILC cells expressing at least one of IL-17A and IL-22) is elevated at the vaccination site of the subject.

29. The method of any one of claims 19 to 26, wherein the expression of one or more cytokines selected from any one of IL-13, IL-4, IL-9, and IL-5 is decreased at the vaccination site of the subject.

30. The method of any one of claims 19 to 28, wherein the expression of IL-13 is decreased at the vaccination site of the subject.

31. The method of claim 28 or claim 29, wherein the cytokine expression is transiently decreased.

32. Use of a first agent comprising an immune stimulator that stimulates or otherwise enhances an immune response to the target antigen or a polynucleotide sequence encoding an immune stimulator that stimulates or otherwise enhances an immune response to the target antigen and a second agent comprising an inhibitor of IL-25 function or a polynucleotide sequence encoding an inhibitor of IL-25 function in the manufacture of a medicament for stimulating an immune response to the target antigen in a subject.

33. Use of a first agent comprising an immune stimulator that stimulates or otherwise enhances an immune response to the target antigen or a polynucleotide sequence encoding an

immune stimulator that stimulates or otherwise enhances an immune response to the target antigen and a second agent comprising an inhibitor of IL-25 function or a polynucleotide sequence encoding an inhibitor of IL-25 function in the manufacture of a medicament for preventing or treating a disease or condition associated with the presence of aberrant expression of the target antigen in a subject.

34. The use of claim 32, wherein the disease or condition is a virus infection or a bacterial infection.

35. The use of claim 33, wherein the virus infection is selected from human immunodeficiency virus (HIV) (*e.g.*, GenBank Accession No. U18552), measles, mumps, rubella, poliomyelitis, hepatitis A, B (*e.g.*, GenBank Accession No. E02707), and C (*e.g.*, GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (*e.g.*, types 4 and 7), rabies (*e.g.*, GenBank Accession No. M34678), yellow fever, Epstein-Barr virus and other herpesviruses such as papillomavirus, Ebola virus, influenza virus, Japanese encephalitis (*e.g.*, GenBank Accession No. E07883), dengue (*e.g.*, GenBank Accession No. M24444), hantavirus, Sendai virus, respiratory syncytial virus, othromyxoviruses, vesicular stomatitis virus, visna virus, and cytomegalovirus.

36. An immunostimulatory antigen-presenting cell or antigen-presenting cell precursor which presents an antigen that corresponds to at least a portion of the target antigen, and which expresses or otherwise produces an inhibitor of IL-25 function.

37. A method for producing an immunostimulatory antigen-presenting cell or antigen-presenting cell precursor, the method comprising contacting an antigen-presenting cell or antigen-presenting cell precursor with (1) an antigen that corresponds to at least a portion of the target antigen or a polynucleotide from which the antigen is expressible for a time and under conditions sufficient for the antigen or a processed form thereof to be presented by the antigen-presenting cell or antigen-presenting cell precursor, and (2) with a composition according to any one of claims 1 to 14, for a time and under conditions for the inhibitor of IL-25 function to be produced by or otherwise provided in the antigen-presenting cell or antigen-presenting cell precursor.

38. A method according to claim 36, wherein the inhibitor of IL-25 function is secreted by the antigen-presenting cell or antigen-presenting cell precursor.

39. A method of eliciting an immune response to a target antigen in a subject, the method comprising: (1) administering a first composition that comprises a first agent that comprises an immune stimulator that stimulates or otherwise enhances an immune response to the target antigen or a polynucleotide sequence encoding an immune stimulator that stimulates or otherwise enhances an immune response to the target antigen; and (2) administering a second composition that comprises the first agent together with a second agent that comprises an inhibitor of IL-25 function or a polynucleotide sequence encoding an inhibitor of IL-25 function.

40. The method of claim 38, wherein the first composition is administered to the subject before the second composition is administered to the subject.

41. The method of claim 38 or claim 39, wherein the second composition is administered to the subject one or more times after the administration of the first composition.

42. The method of any one of claims 38 to 40, wherein the first composition is administered locally, and the second composition is administered systemically.

43. The method of any one of claims 38 to 41, wherein the first composition is administered intranasally.

44. The method of any one of claims 38 to 42, wherein the second composition is administered intramuscularly.

45. The method of any one of claims 38 to 43, wherein the first composition further comprises an inhibitor of IL-4 function or a polynucleotide sequence encoding an inhibitor of IL-4 function.

46. The method of claim 44, wherein the inhibitor of IL-4 function is a IL-4C123 polypeptide.

47. The method of claim 44, wherein the polynucleotide sequence encoding an inhibitor comprises the nucleic acid sequence set forth in SEQ ID NO: 101.

48. A method for enhancing mucosal immunity to a target antigen in a subject that is administered a first agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to the target antigen, the method comprising systemically administering to the subject a second agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible, to thereby enhance mucosal immunity to the target antigen.

49. The method of claim 48, wherein the first agent and the second agent are systemically administered concurrently to the subject.

50. The method of claim 48 or claim 49, wherein ther systemic administration is intramuscular administration.

51. Use of an IL-25 inhibitory agent comprising an inhibitor of IL-25 function or a polynucleotide from which a nucleotide sequence encoding an inhibitor of IL-25 function is expressible, for enhancing mucosal immunity to a target antigen by an immunostimulatory agent comprising an immune stimulator or a polynucleotide sequence from which a nucleotide sequence encoding an immune stimulator is expressible, wherein the immune stimulator stimulates or otherwise enhances an immune response to the target antigen, wherein the IL-25 inhibitory agent is formulated for systemic admninistration.

52. The use of claim 51, wherein the IL-25 inhibitory agent and the immunostimulatory agent are formulated for concurrent administration via a systemic route.

53. The use of claim 51 or claim 52, wherein the systemic administration is intramuscular administration.

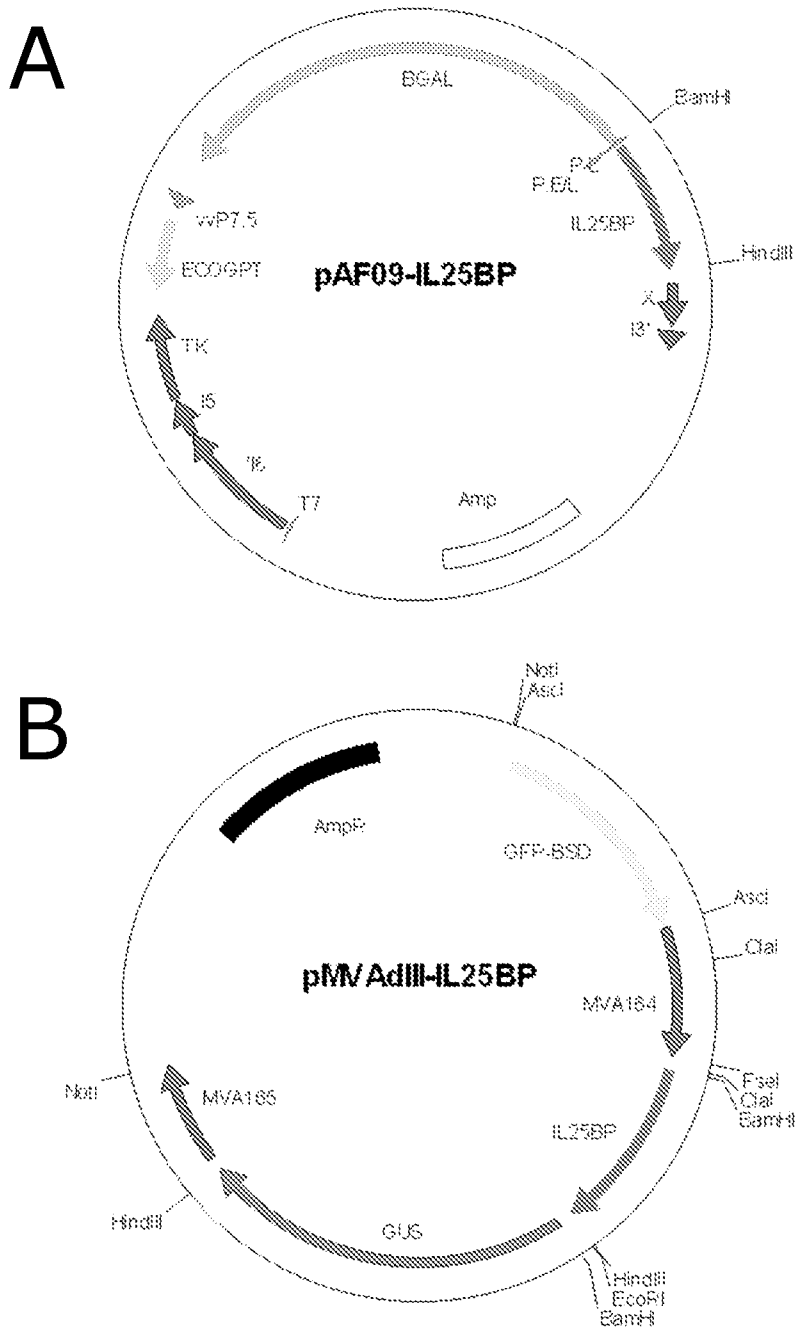
A
BGAL
BamHI
P2
P.EL
IL25BP
HindIII
vvP7.5
ECOGPT
pAF09-IL25BP
I3'
TK
I5
I1
T7
Amp
B
NotI
AscI
AmpR
GFP-BSD
AscI
ClaI
pMVAdIII-IL25BP
MVA184
FseI
ClaI
BamHI
NotI
MVA185
IL25BP
HindIII
GUS
HindIII
EcoRI
BamHI

FIGURE 1

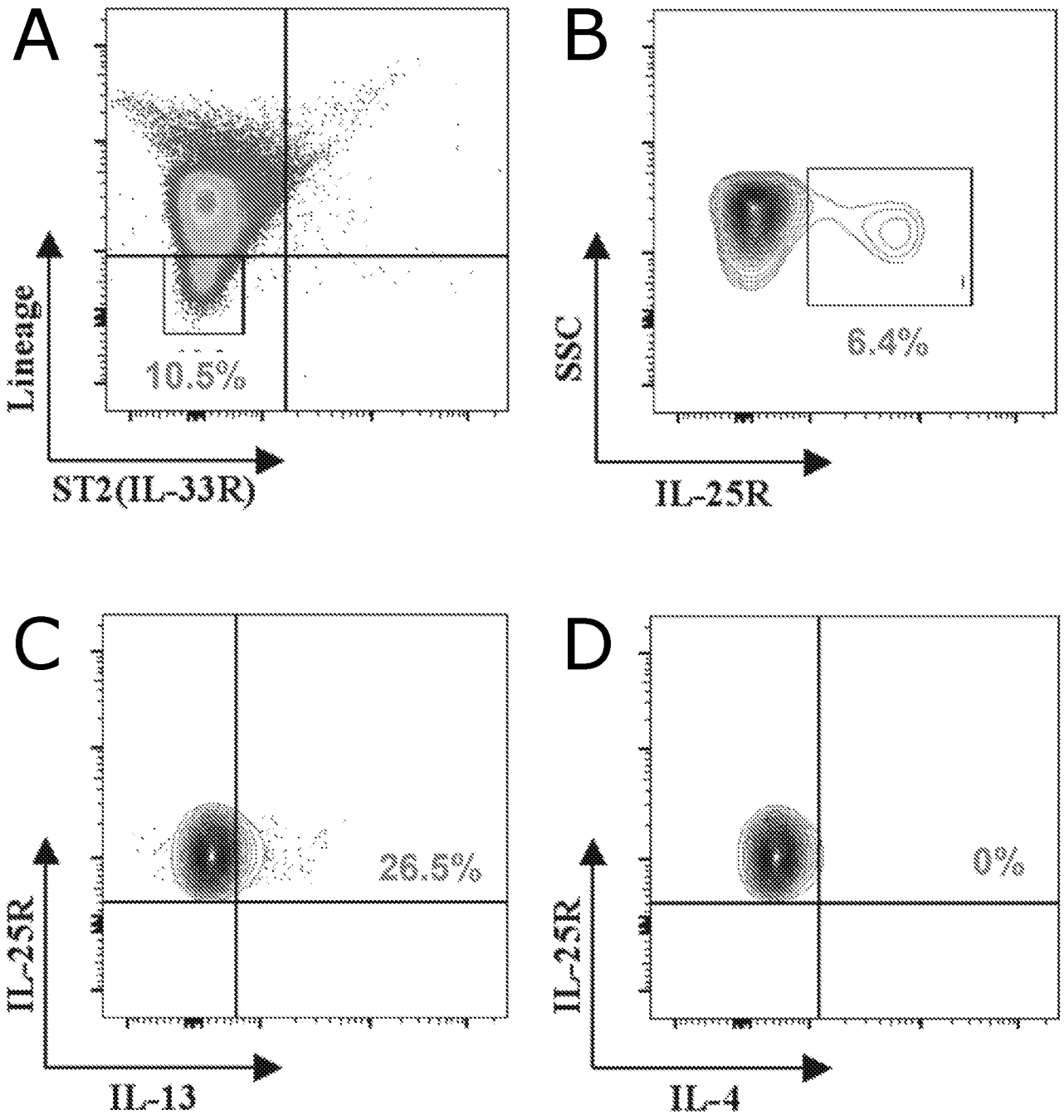
A
Lineage
ST2(IL-33R)
10.5%
B
SSC
IL-25R
6.4%
C
IL-25R
IL-13
26.5%
D
IL-25R
IL-4
0%

FIGURE 2

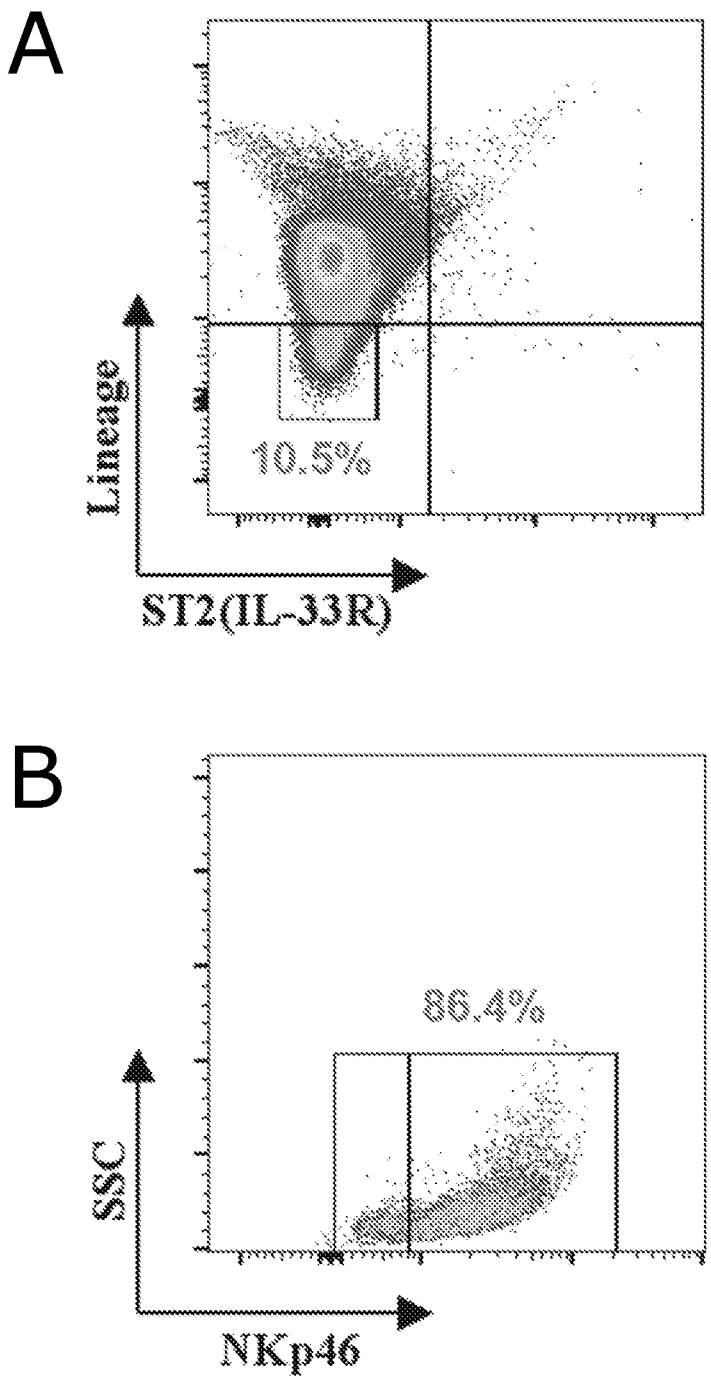

FIGURE 3

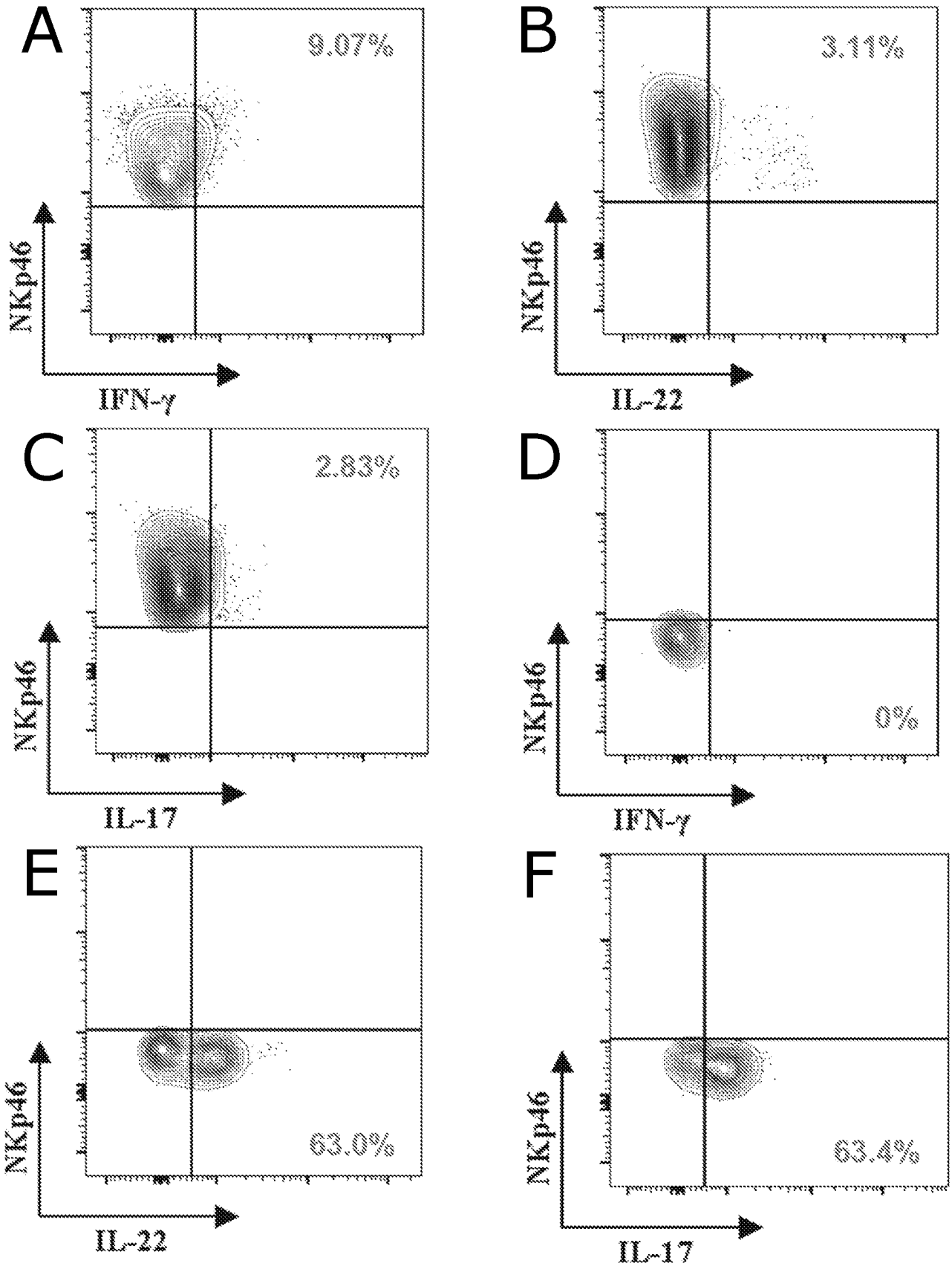
A
9.07%
NKp46
IFN-γ
B
3.11%
NKp46
IL-22
C
2.83%
NKp46
IL-17
D
0%
NKp46
IFN-γ
E
63.0%
NKp46
IL-22
F
63.4%
NKp46
IL-17

FIGURE 4

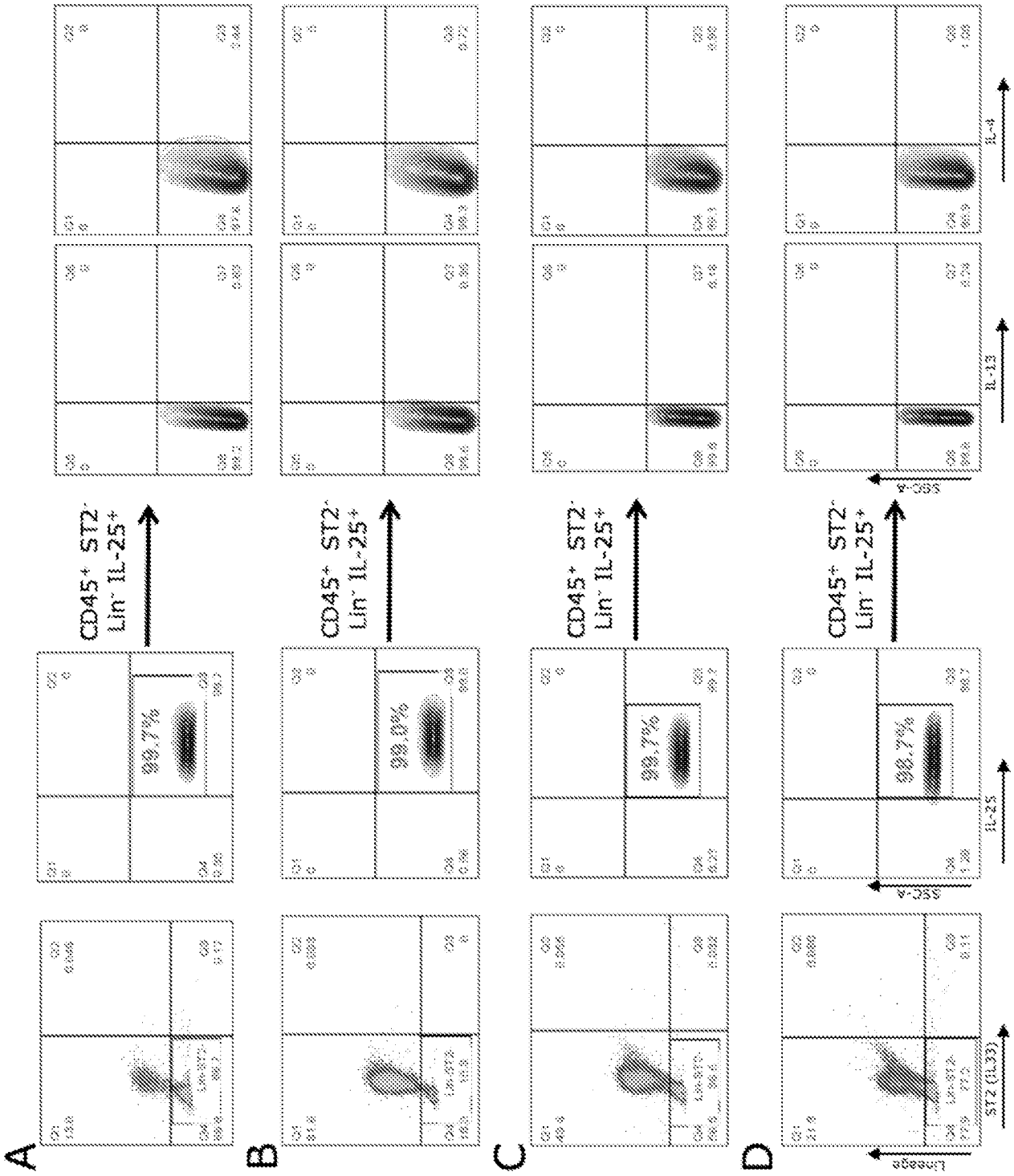
A
B
C
D
CD45+ ST2- Lin- IL-25+
99.7%
99.8%
99.7%
99.7%
IL-25
IL-13
IL-4
ST2 (IL33)
Lineage

FIGURE 5

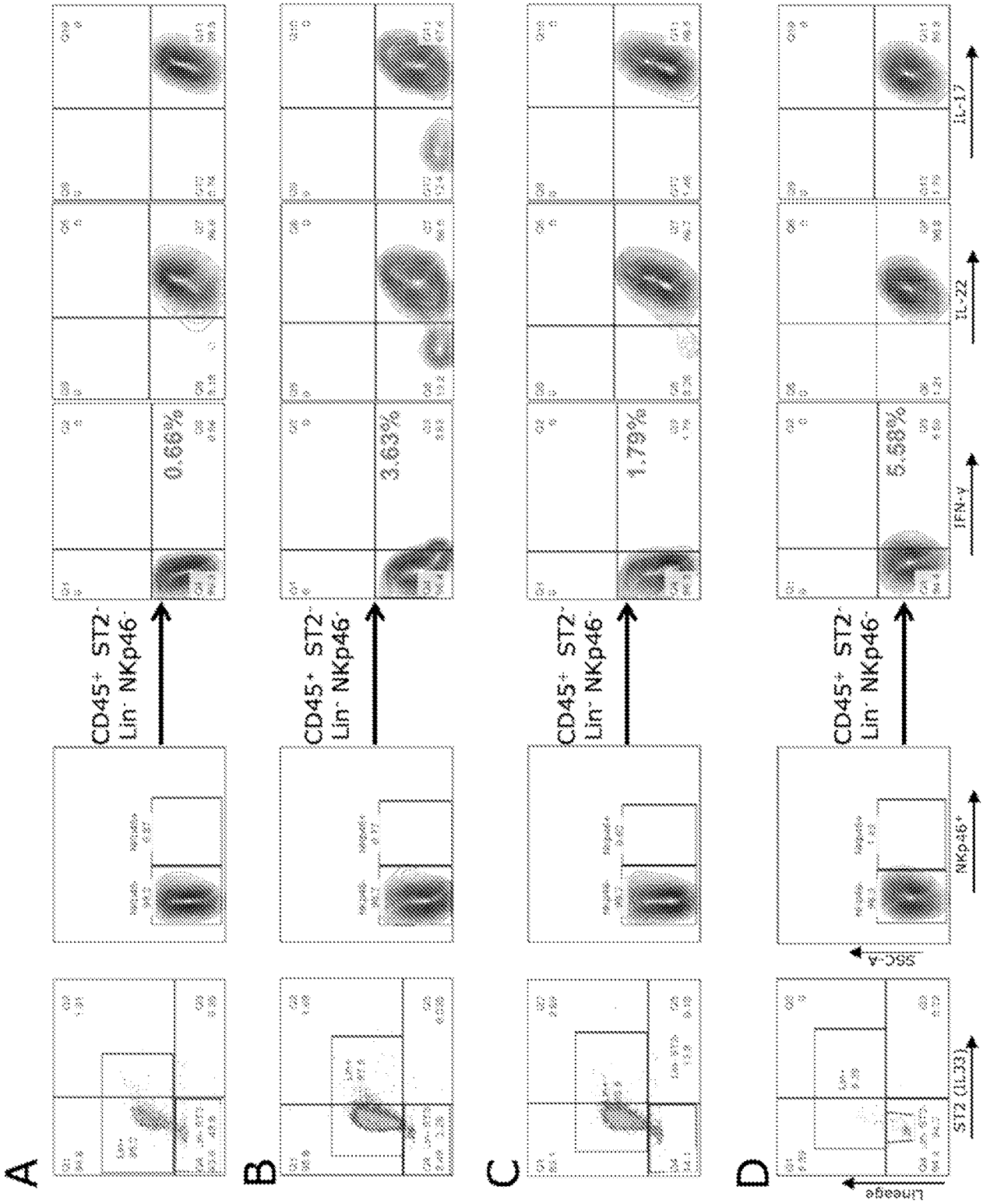
A
B
C
D
CD45+ ST2- Lin- NKp46-
0.66%
3.63%
1.79%
5.68%
IFN-γ
IL-22
IL-17
NKp46+
SSC-A
Lineage
ST2 (IL33)

FIGURE 6

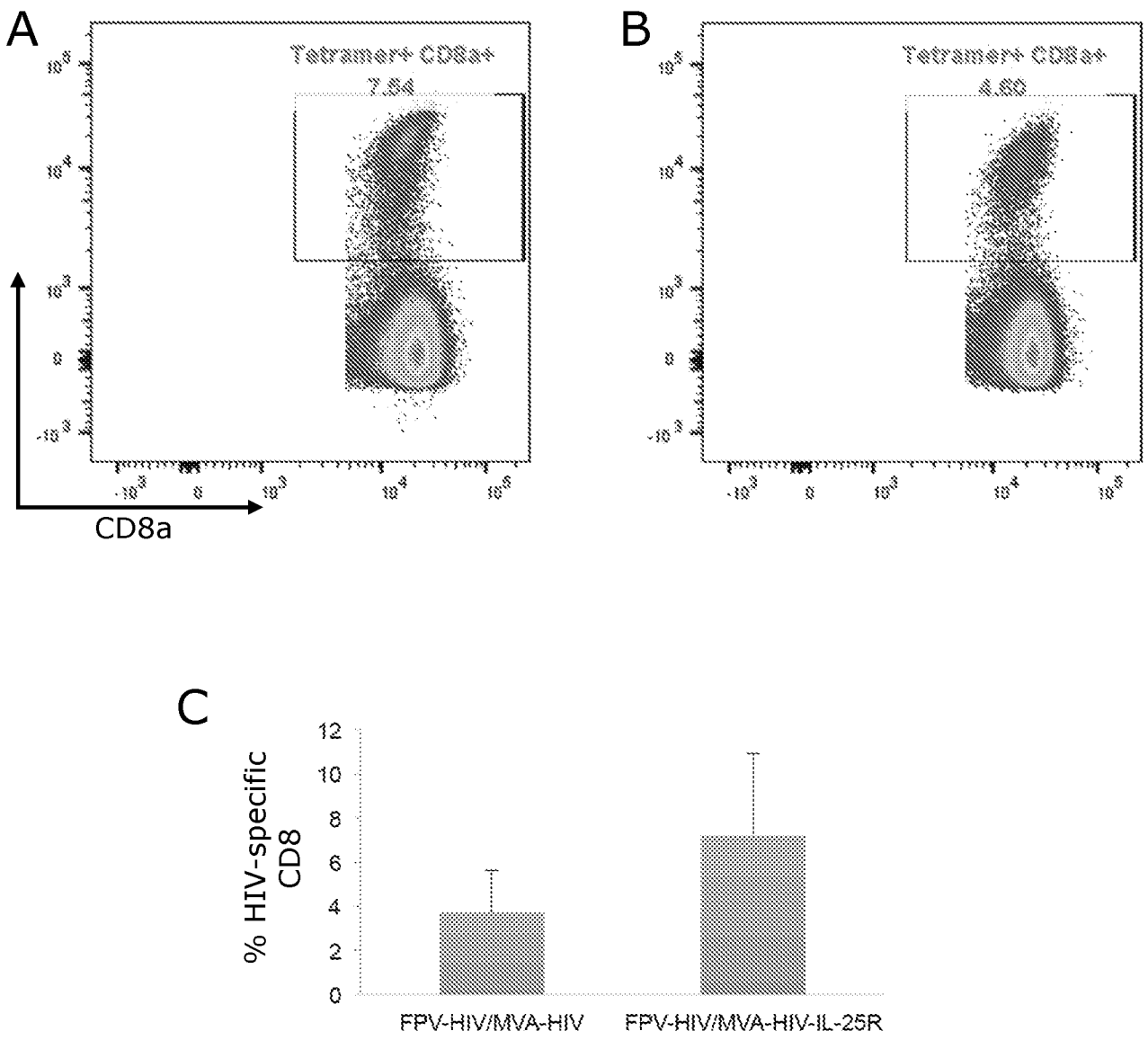
A
Tetramer+ CD8a+
7.84
CD8a
B
Tetramer+ CD8a+
4.80
C
% HIV-specific CD8
12
10
8
6
4
2
0
FPV-HIV/MVA-HIV
FPV-HIV/MVA-HIV-IL-25R

FIGURE 7

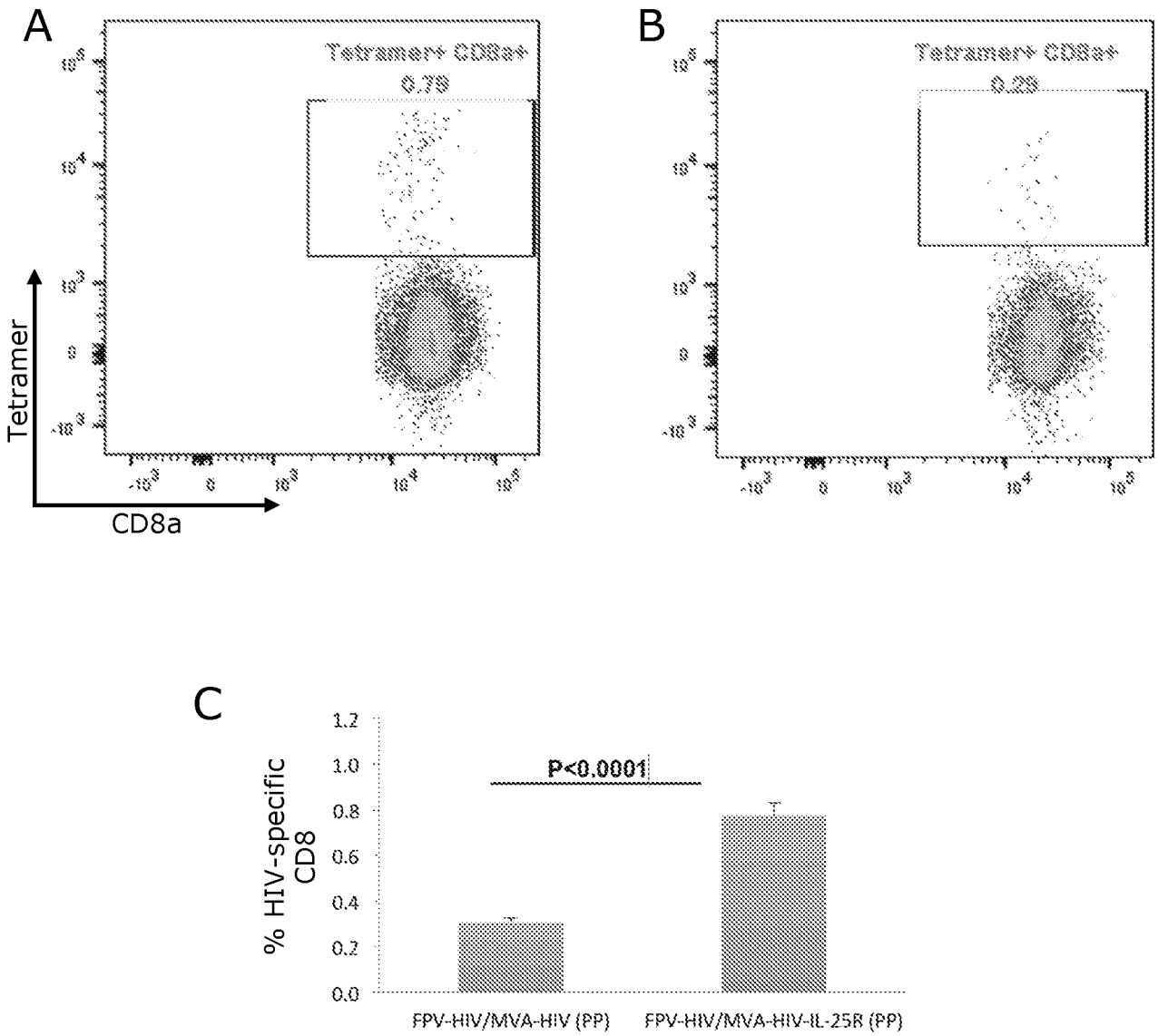
A
Tetramer+ CD8a+
0.79
Tetramer
CD8a
B
Tetramer+ CD8a+
0.29
C
% HIV-specific CD8
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P<0.0001
FPV-HIV/MVA-HIV (PP)
FPV-HIV/MVA-HIV-IL-25R (PP)

FIGURE 8

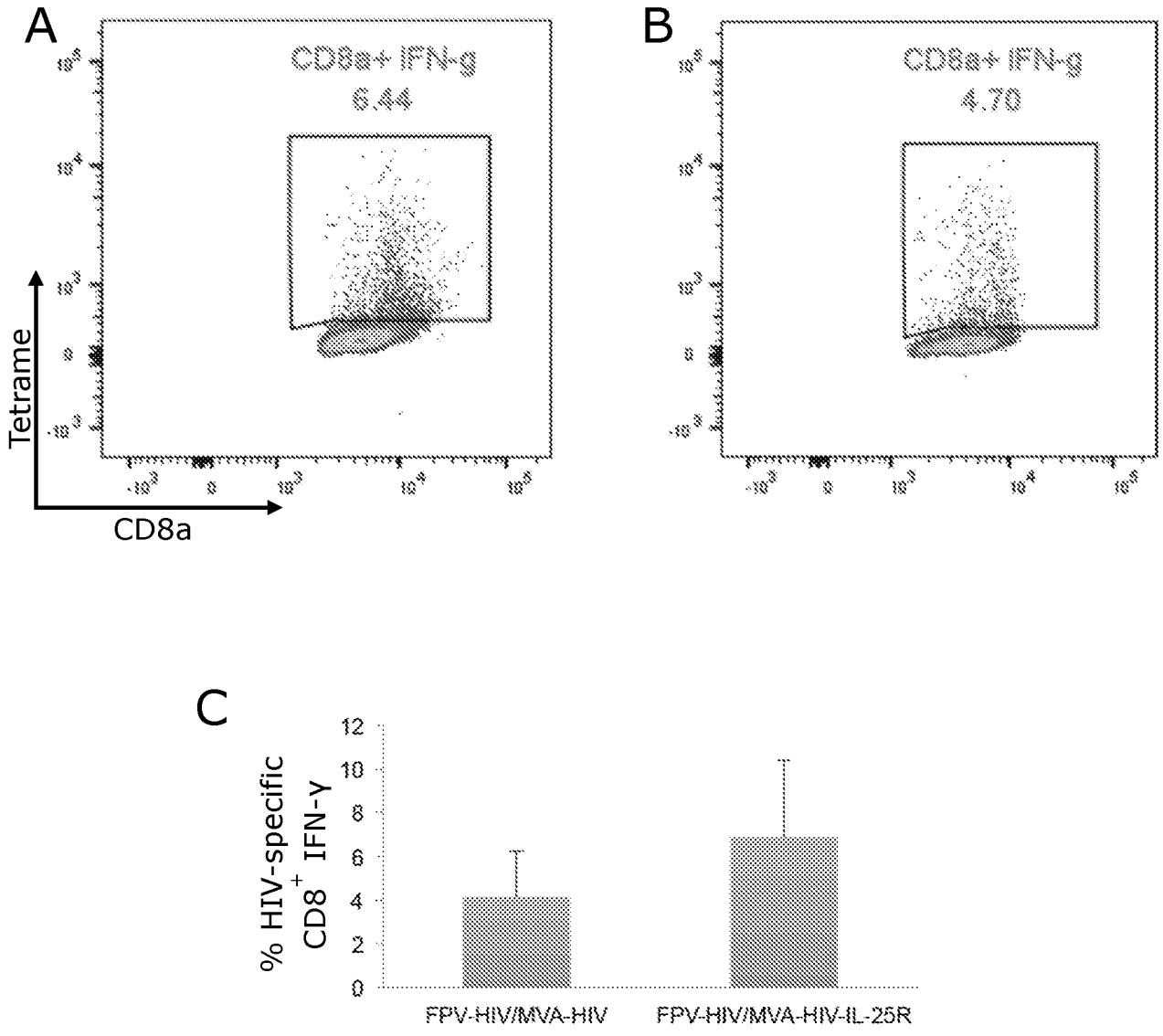
A
CD8a+ IFN-g
6.44
Tetrame
CD8a
B
CD8a+ IFN-g
4.70
C
% HIV-specific CD8+ IFN-γ
12
10
8
6
4
2
0
FPV-HIV/MVA-HIV
FPV-HIV/MVA-HIV-IL-25R

FIGURE 9

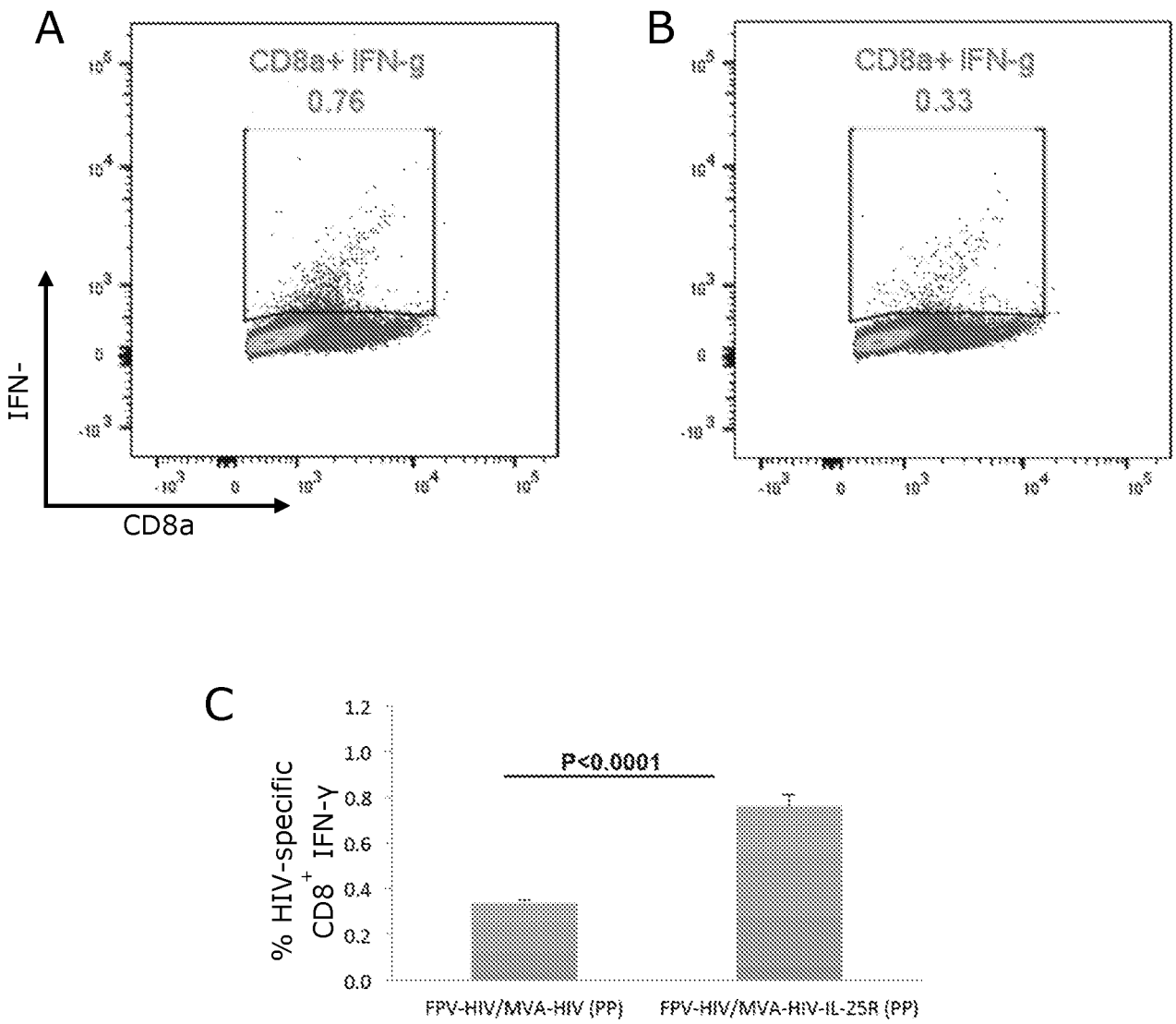
A
CD8a+ IFN-g
0.76
IFN-
CD8a
B
CD8a+ IFN-g
0.33
C
% HIV-specific CD8+ IFN-γ
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P<0.0001
FPV-HIV/MVA-HIV (PP)
FPV-HIV/MVA-HIV-IL-25R (PP)

FIGURE 10

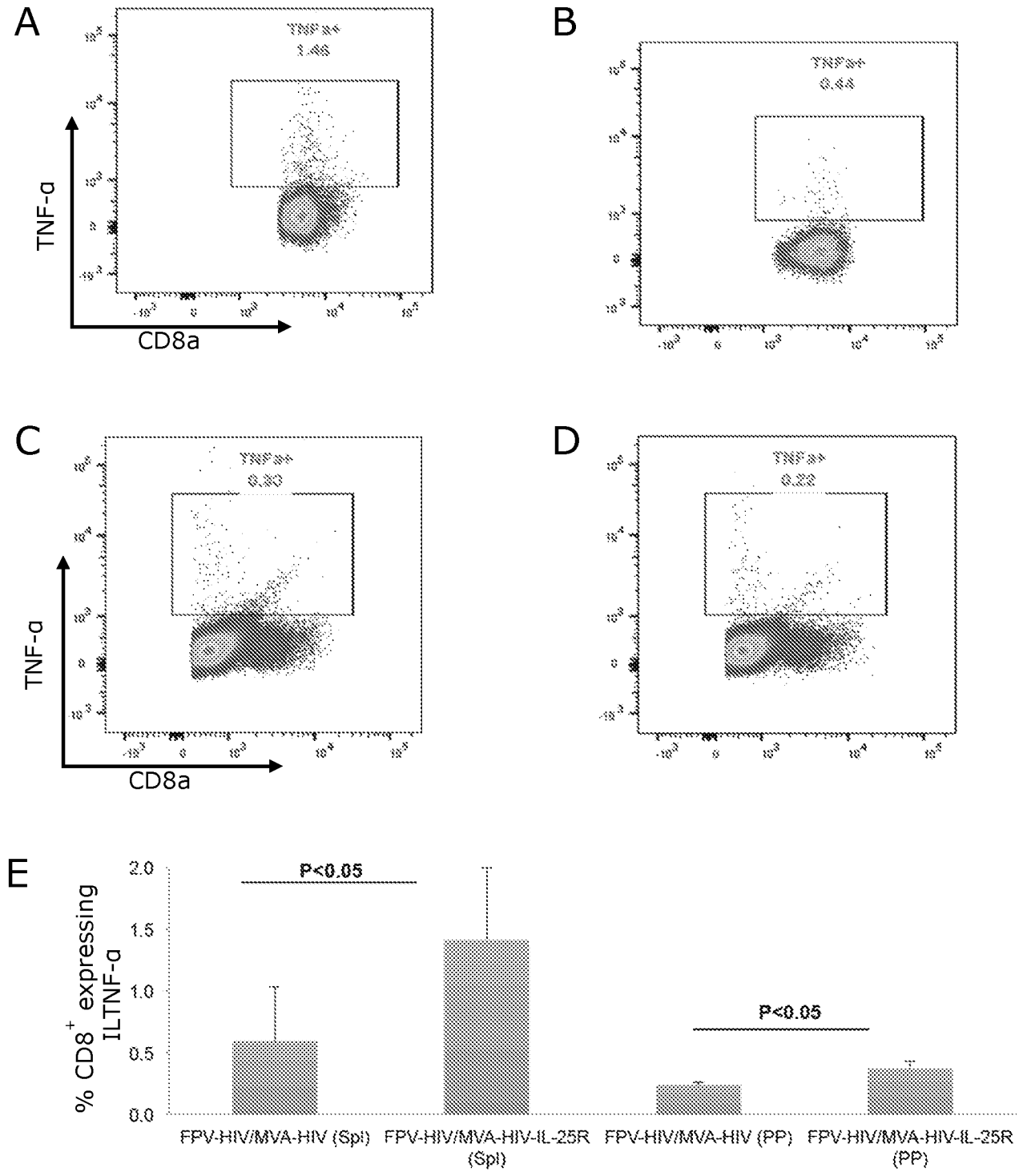
A
TNF a+
1.46
TNF-a
CD8a
B
TNF a+
0.44
C
TNF a+
0.30
TNF-a
CD8a
D
TNF a+
0.22
E
% CD8+ expressing ILTNF-a
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.05
FPV-HIV/MVA-HIV (Spl)
FPV-HIV/MVA-HIV-IL-25R (Spl)
FPV-HIV/MVA-HIV (PP)
FPV-HIV/MVA-HIV-IL-25R (PP)

FIGURE 11

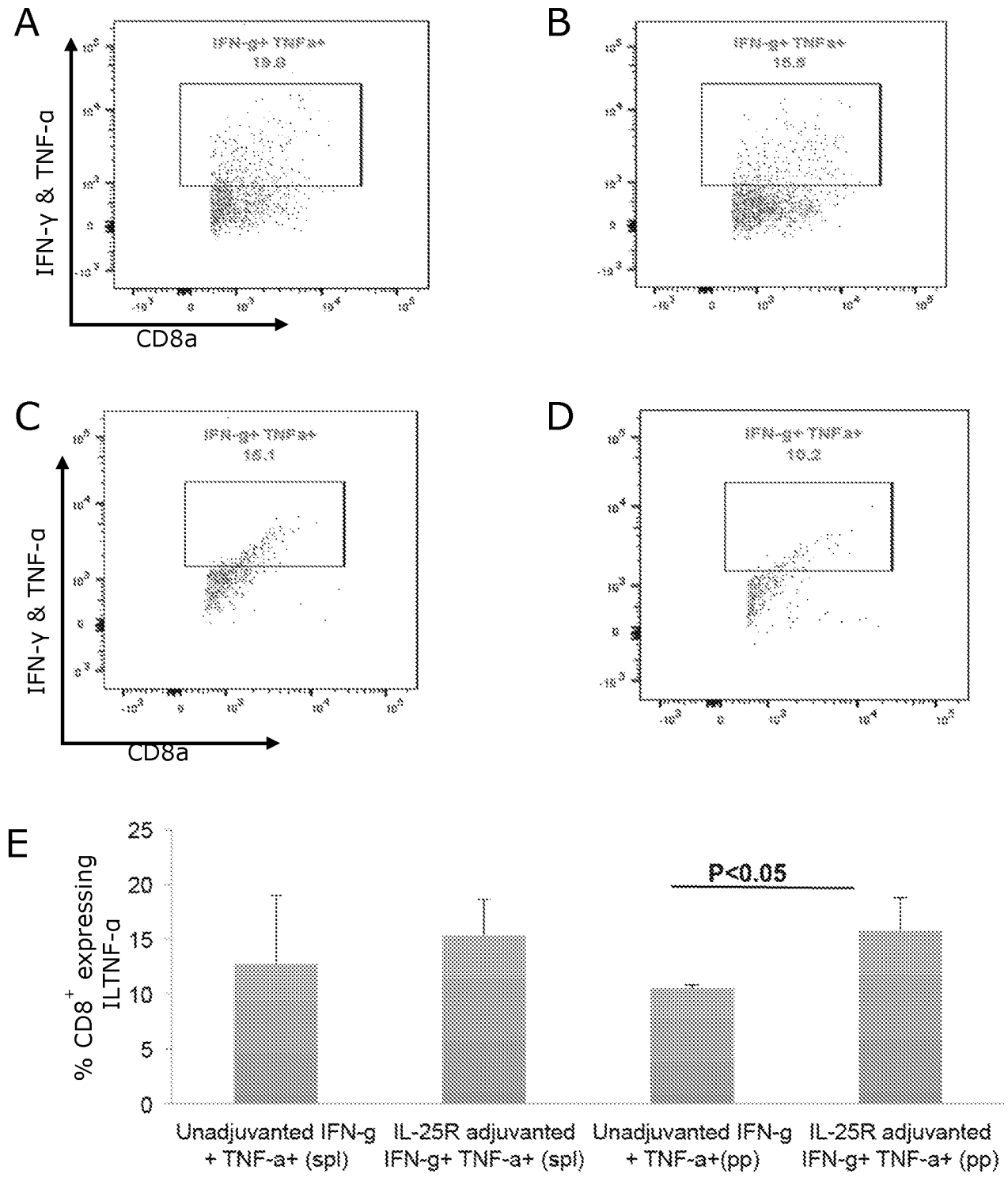
A
B
C
D
E
IFN-γ & TNF-a
CD8a
% CD8+ expressing ILTNF-a
25
20
15
10
5
0
P<0.05
Unadjuvanted IFN-g + TNF-a+ (spl)
IL-25R adjuvanted IFN-g+ TNF-a+ (spl)
Unadjuvanted IFN-g + TNF-a+(pp)
IL-25R adjuvanted IFN-g+ TNF-a+ (pp)

FIGURE 12

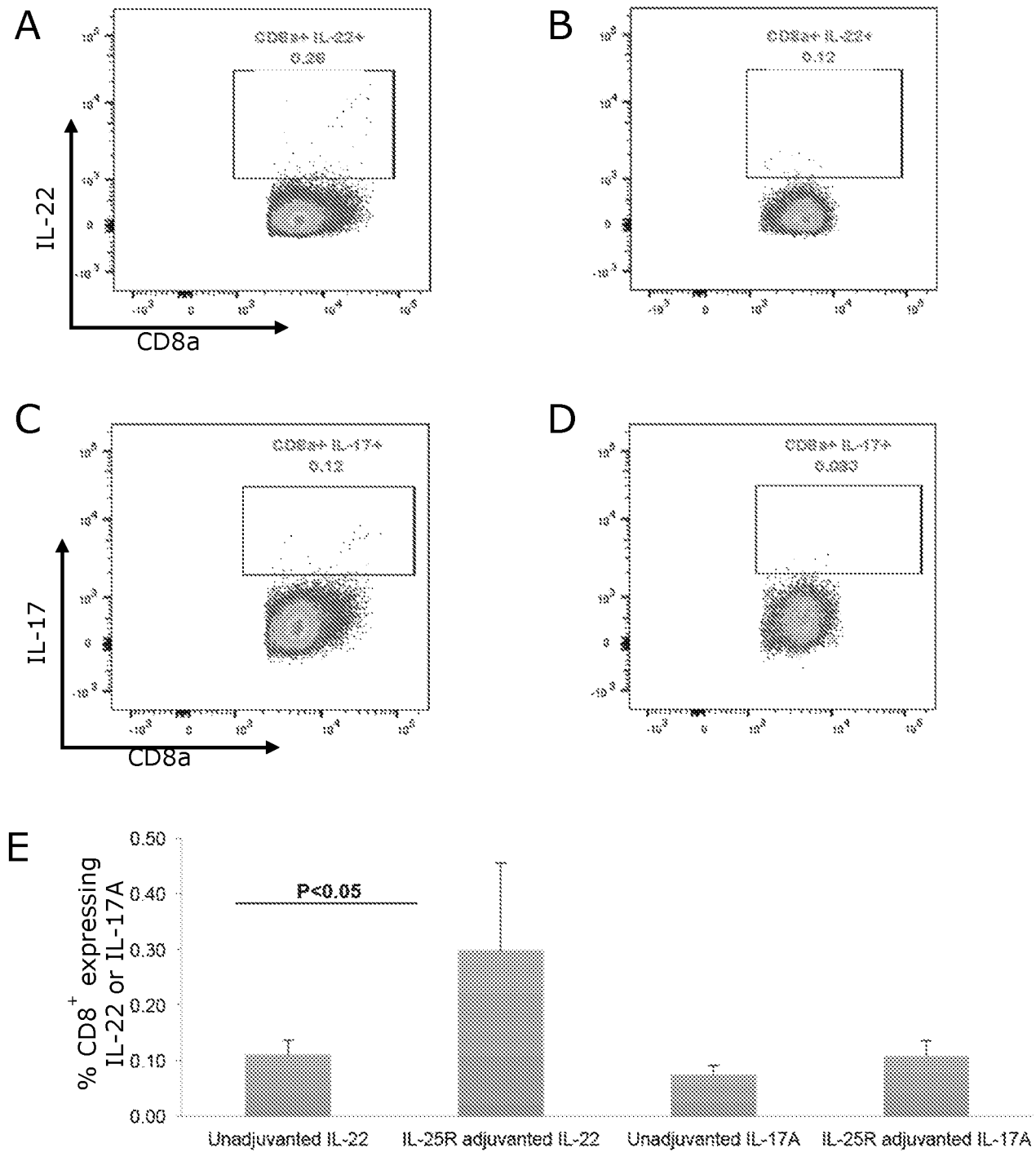
A
B
C
D
E
IL-22
CD8a
IL-17
% CD8+ expressing IL-22 or IL-17A
P<0.05
0.50
0.40
0.30
0.20
0.10
0.00
Unadjuvanted IL-22
IL-25R adjuvanted IL-22
Unadjuvanted IL-17A
IL-25R adjuvanted IL-17A

FIGURE 13

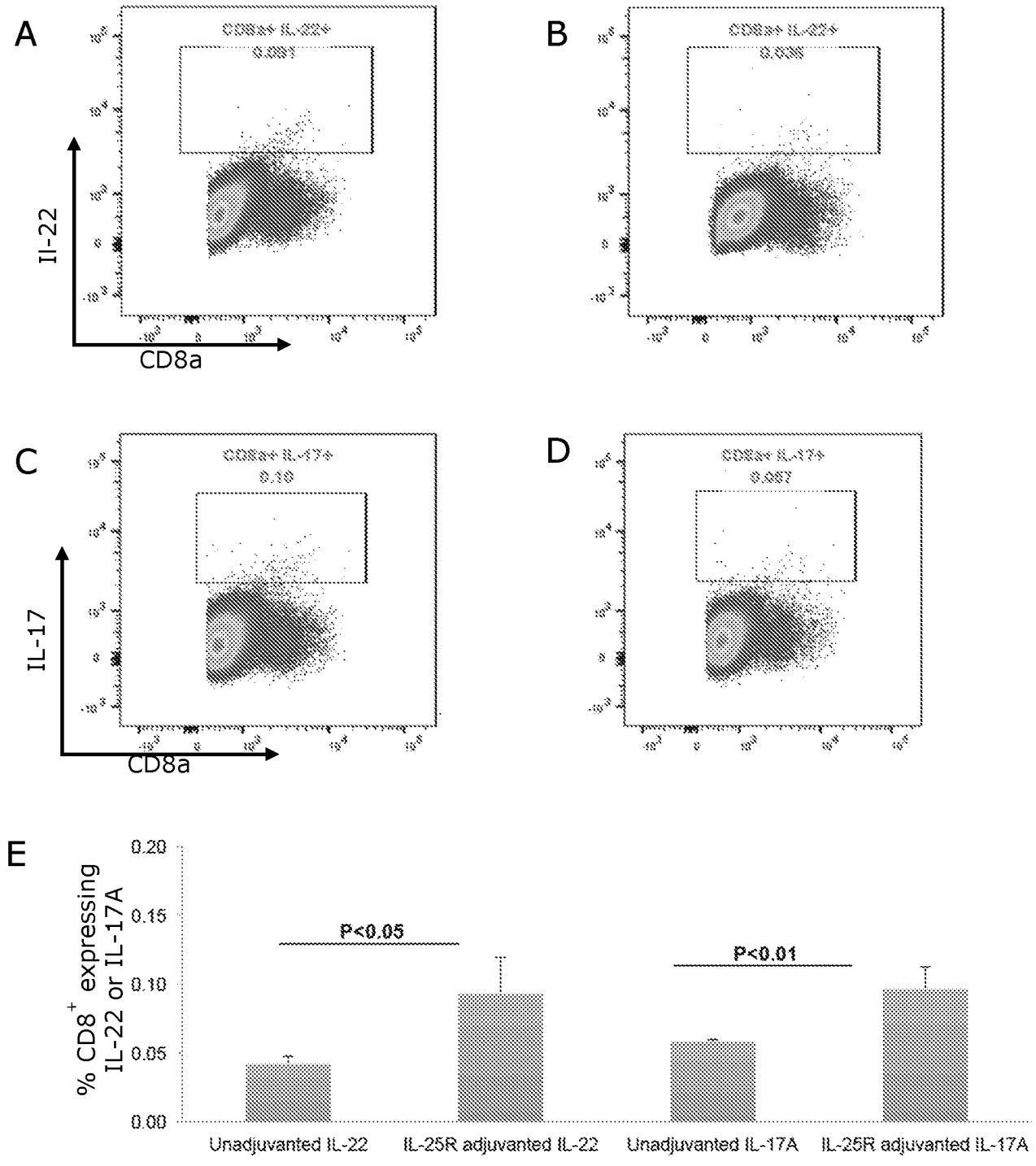
A
B
C
D
E
Il-22
CD8a
IL-17
% CD8+ expressing IL-22 or IL-17A
0.20
0.15
0.10
0.05
0.00
P<0.05
P<0.01
Unadjuvanted IL-22
IL-25R adjuvanted IL-22
Unadjuvanted IL-17A
IL-25R adjuvanted IL-17A

FIGURE 14

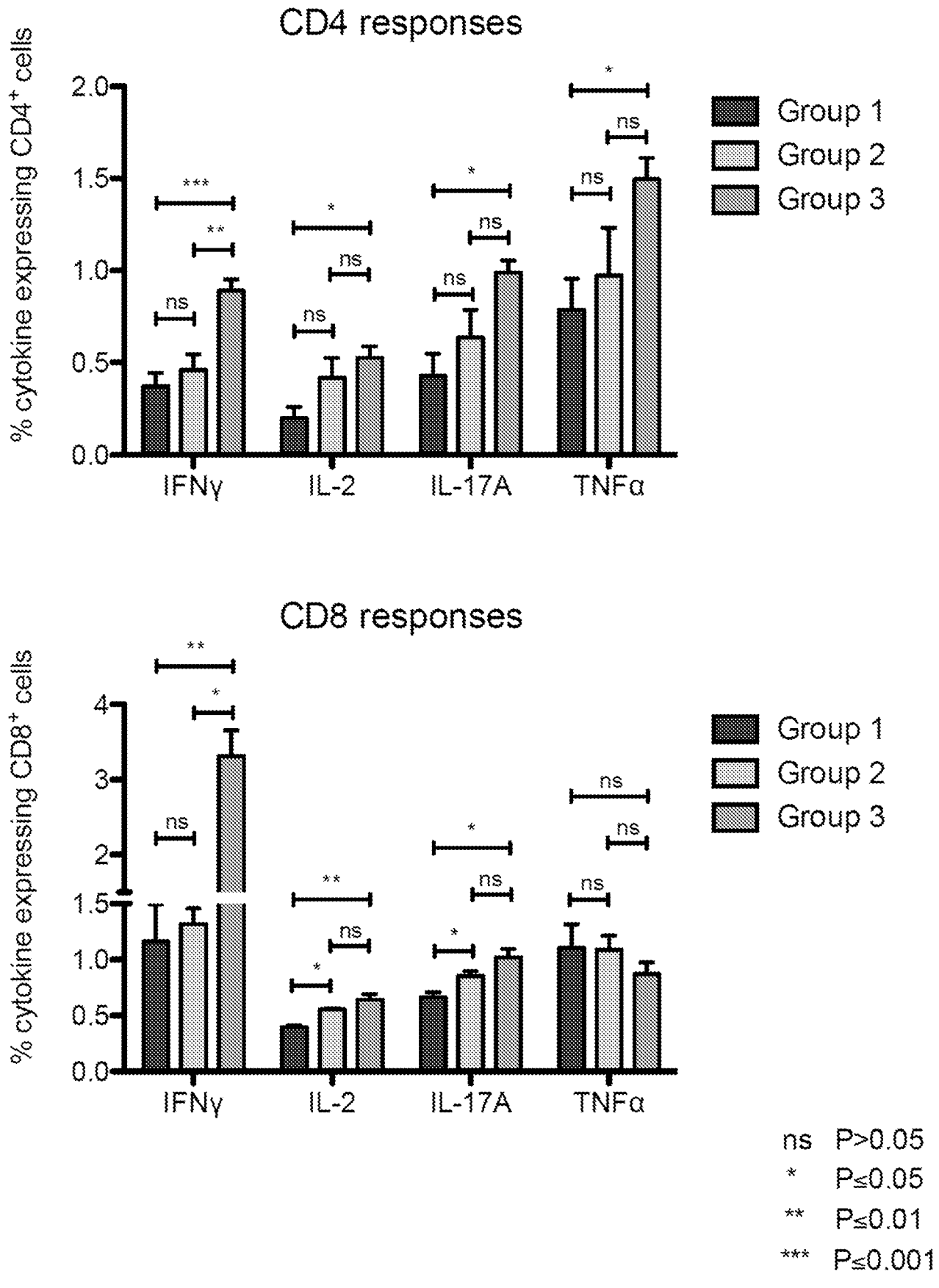
CD4 responses
% cytokine expressing CD4+ cells
2.0
1.5
1.0
0.5
0.0
IFNγ
IL-2
IL-17A
TNFα
Group 1
Group 2
Group 3
CD8 responses
% cytokine expressing CD8+ cells
4
3
2
1.5
1.0
0.5
0.0
IFNγ
IL-2
IL-17A
TNFα
Group 1
Group 2
Group 3
ns P>0.05
* P≤0.05
** P≤0.01
*** P≤0.001

FIGURE 15

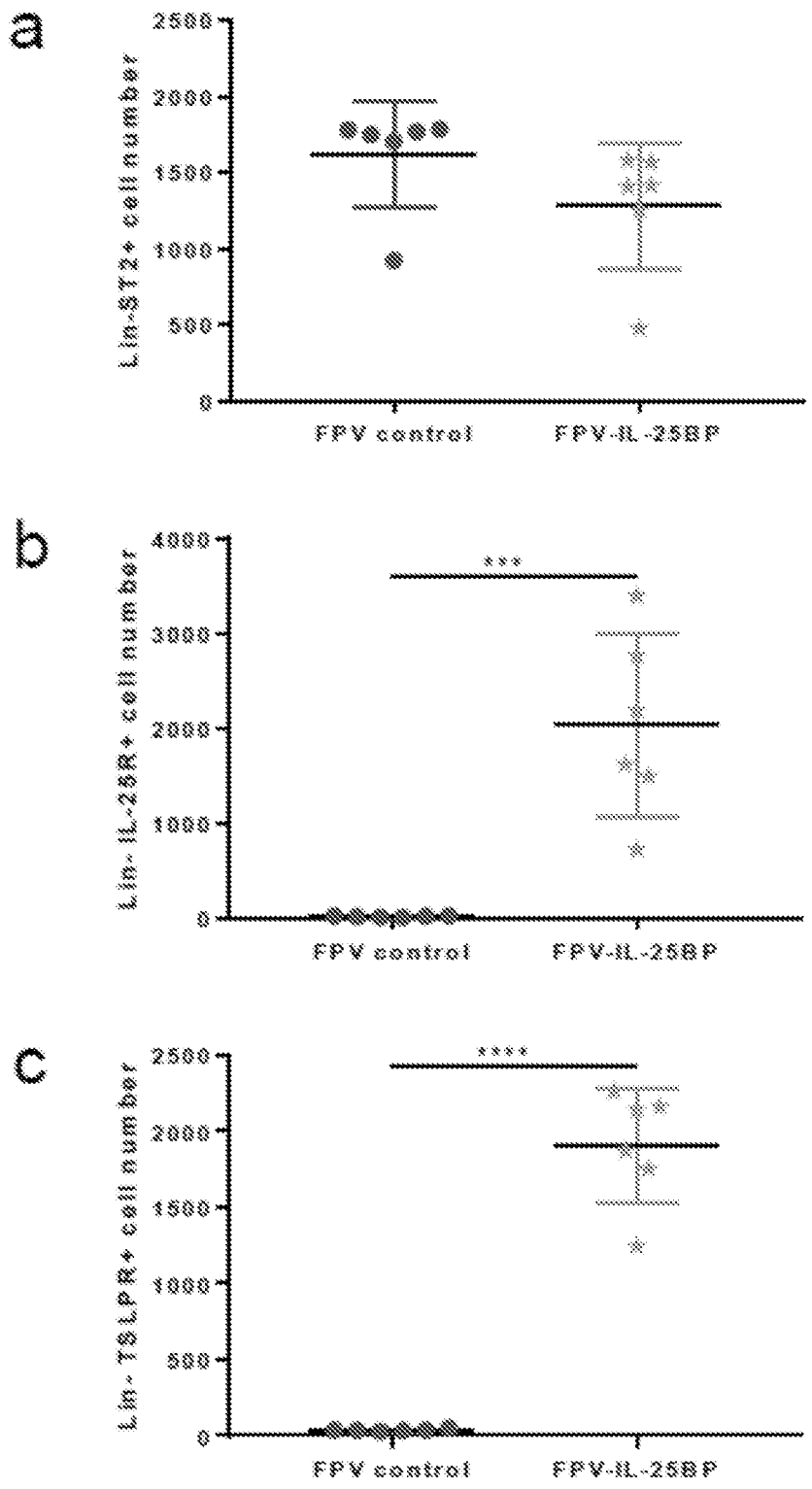
a
Lin-ST2+ cell number
2500
2000
1500
1000
500
0
FPV control
FPV-IL-25BP
b
Lin- IL-25R+ cell number
4000
3000
2000
1000
0
***
FPV control
FPV-IL-25BP
c
Lin- TSLPR+ cell number
2500
2000
1500
1000
500
0
****
FPV control
FPV-IL-25BP

FIGURE 16

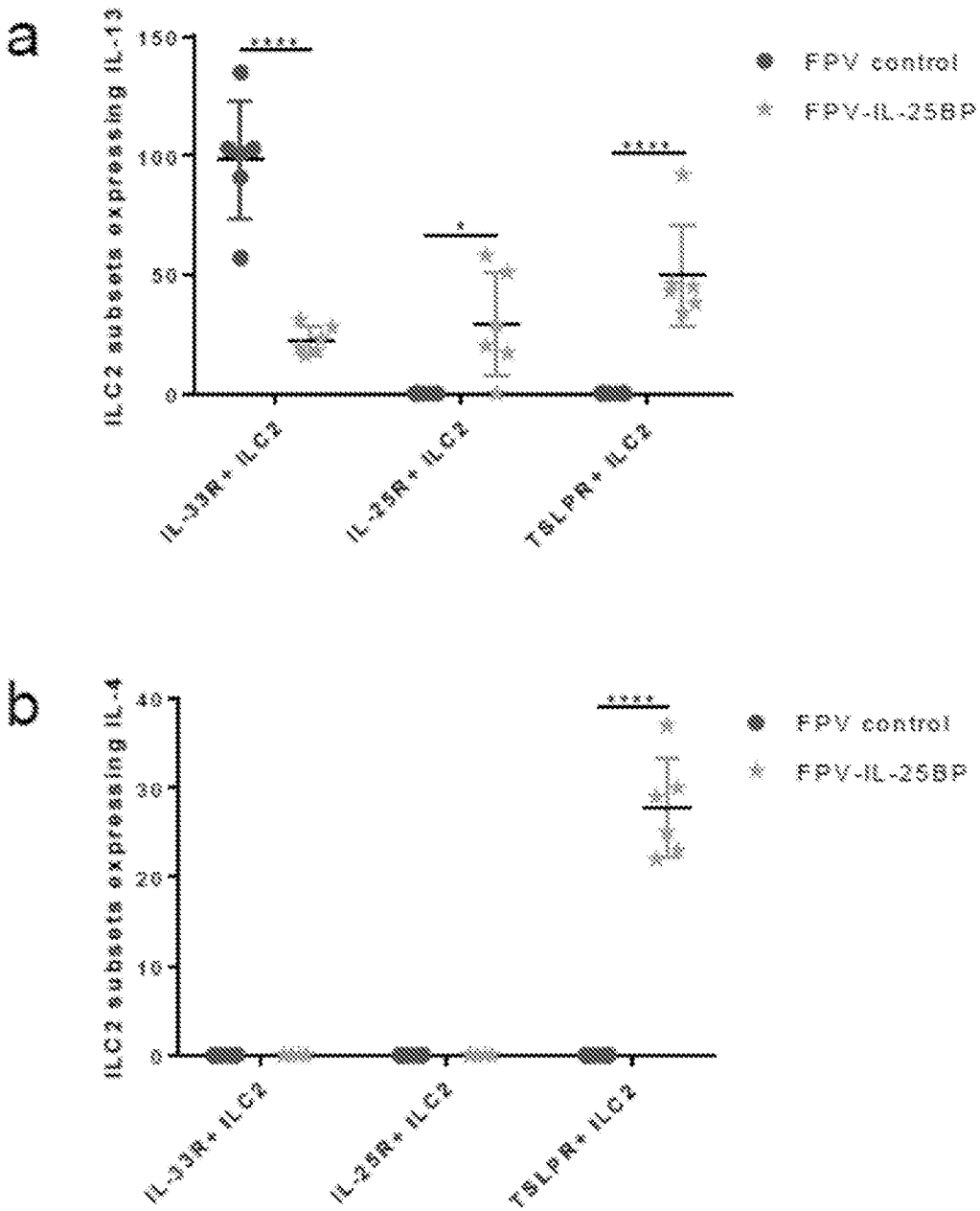
a
ILC2 subsets expressing IL-13
150
100
50
0
****
*
****
IL-33R+ ILC2
IL-25R+ ILC2
TSLPR+ ILC2
FPV control
FPV-IL-25BP
b
ILC2 subsets expressing IL-4
40
30
20
10
0
****
IL-33R+ ILC2
IL-25R+ ILC2
TSLPR+ ILC2
FPV control
FPV-IL-25BP

FIGURE 17

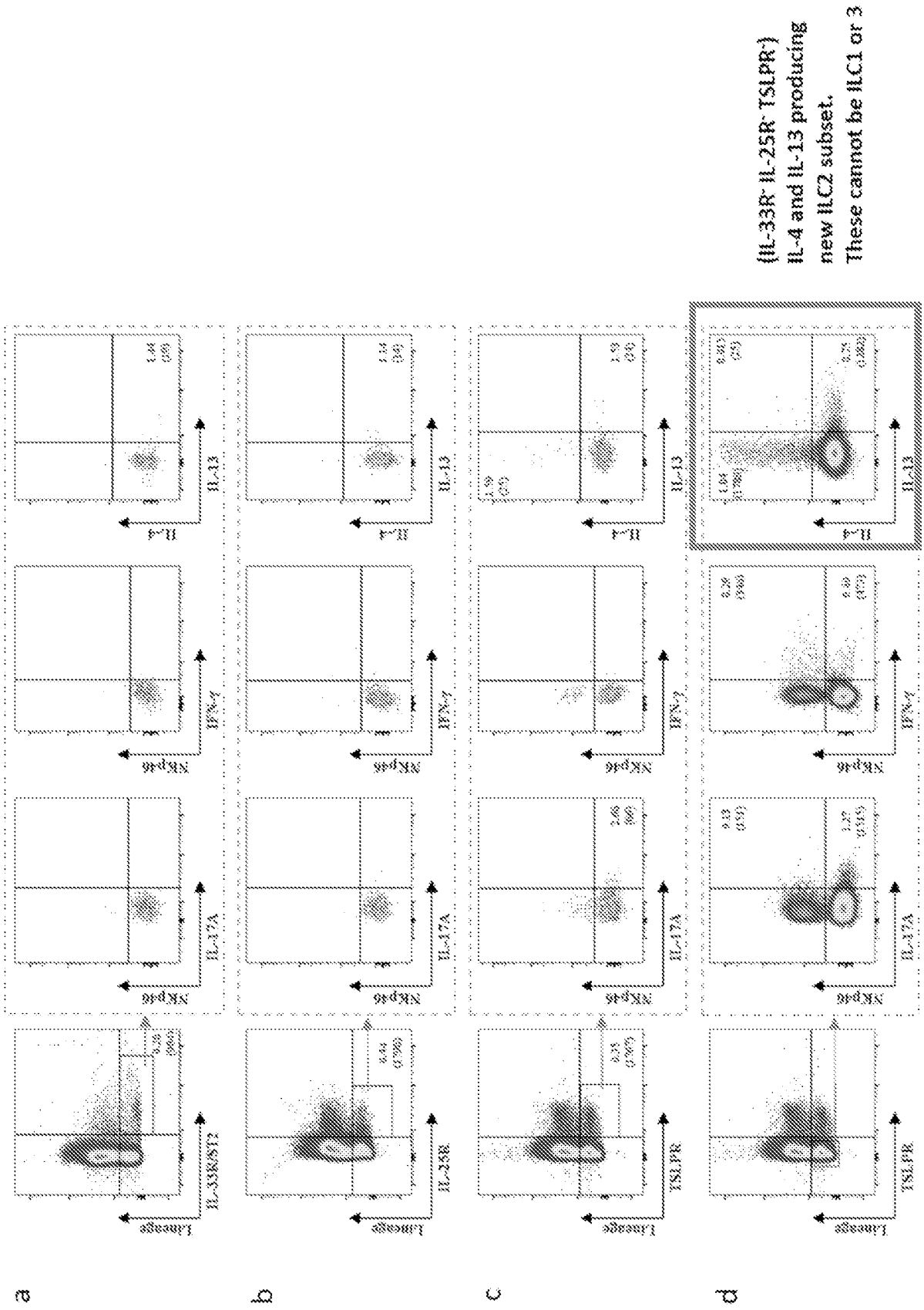
{IL-33R- IL-25R- TSLPR-}
IL-4 and IL-13 producing
new ILC2 subset.
These cannot be ILC1 or 3
a
b
c
d
Lineage
IL-33R/ST2
IL-25R
TSLPR
NKp46
IL-17A
IFN-γ
IL-13
IL-4

FIGURE 18

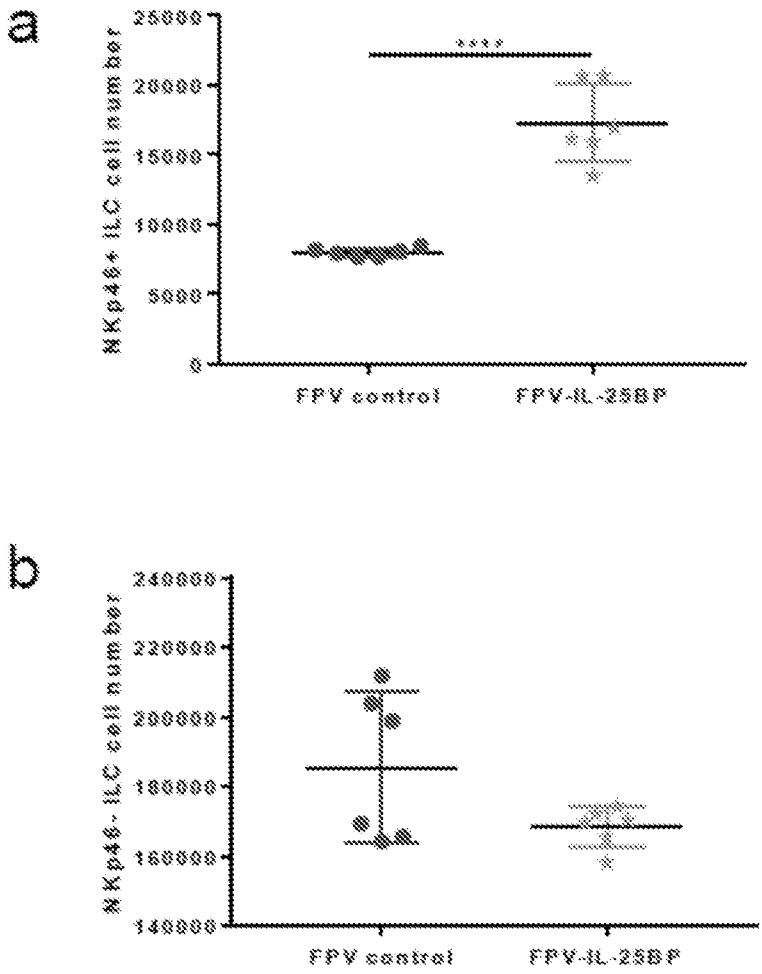
a
NKp46+ ILC cell number
250000
200000
150000
100000
50000
0
****
FPV control
FPV-IL-25BP
b
NKp46- ILC cell number
240000
220000
200000
180000
160000
140000
FPV control
FPV-IL-25BP

FIGURE 19

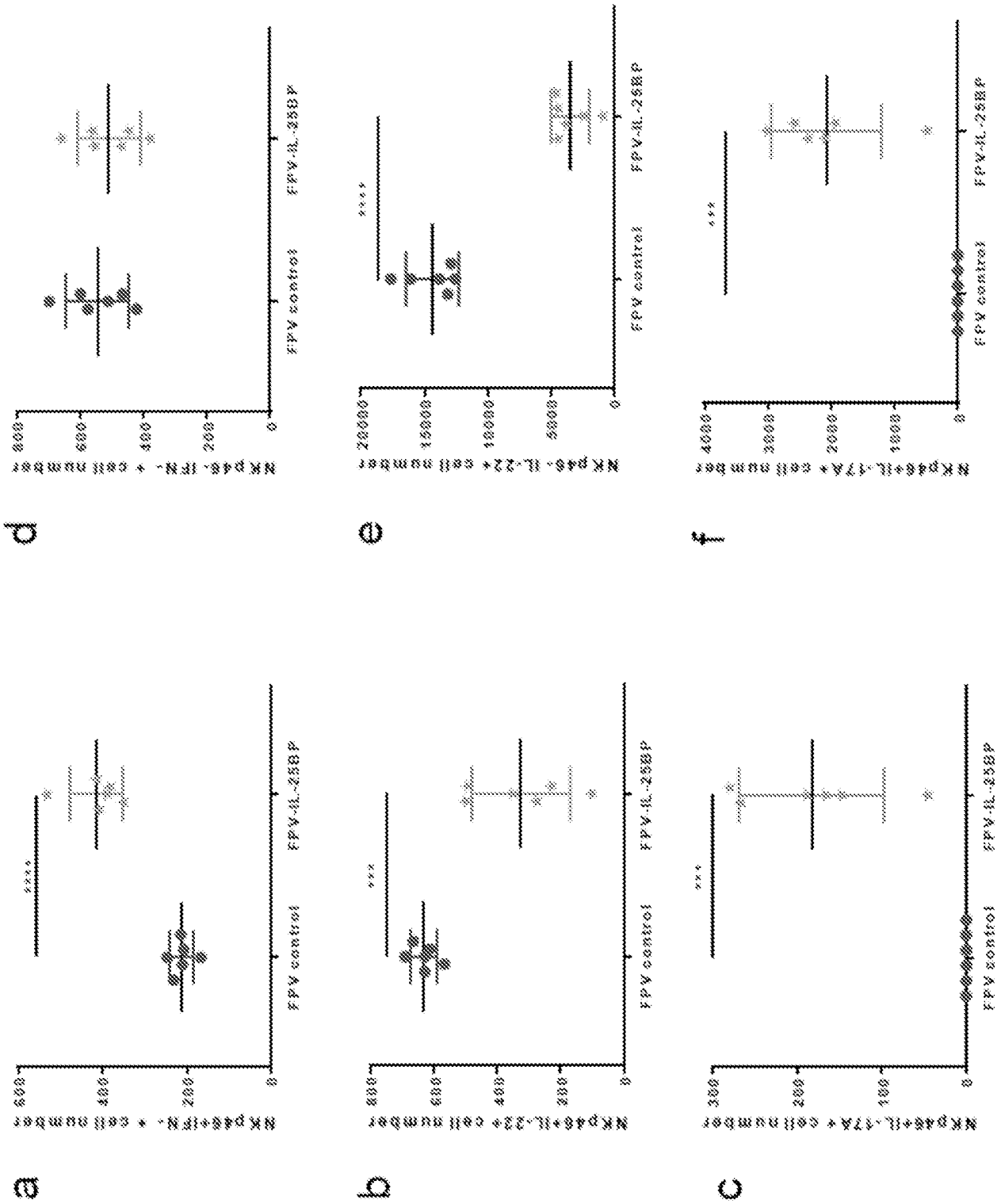
a
NKp46+IFN- + cell number
FPV control
FPV-IL-25BP
****
b
NKp46+IL-23+ cell number
FPV control
FPV-IL-25BP
***
c
NKp46+IL-17A+ cell number
FPV control
FPV-IL-25BP
***
d
NKp46- IFN- + cell number
FPV control
FPV-IL-25BP
e
NKp46- IL-22+ cell number
FPV control
FPV-IL-25BP
****
f
NKp46+IL-17A+ cell number
FPV control
FPV-IL-25BP
***

FIGURE 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2017/050772

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/39 (2006.01) ***A61K 39/00 (2006.01)*** ***A61K 39/12 (2006.01)*** ***A61K 39/02 (2006.01)*** ***A61K 39/04 (2006.01)*** ***A61K 38/20 (2006.01)*** ***A61P 37/02 (2006.01)*** ***A61P 37/04 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: EPOQUE: PATENW; STN: HCAPLUS, MEDLINE, EMBASE and BIOSIS; PUBLIC: ESPACENET, PATENTSCOPE and PUBMED.

CPC/IPC marks: A61K39/39. Keywords: Interleukin 25, Interleukin 17E, IL25BP, IL17RA, IL17RB, inhibitor, antagonist, adjuvant, immunostimulatory, antigen, vaccine, HIV, tuberculosis, T cells, Antigen presenting cells, cytotoxic T lymphocytes, innate lymphoid cells, mucosal immunity and like terms.

Applicant and inventor name searches were carried out using Internal IPA databases PAMSNOSE and INTESS and public databases ESPACENET, PATENTSCOPE and PUBMED.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2017	21 September 2017
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Holly Thomas AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256157

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2017/050772**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2015/031778 A2 (GLOBEIMMUNE, INC. et al.) 05 March 2015 Abstract; [0015]; [00132]-[00133]; [00140] Whole document.	1-18 19-53
A	US 2012/0064073 A1 (CHEN J. et al.) 15 March 2012 Abstract; [0048]-[0052]; Examples.	1-53
A	HEPWORTH et al. "Regulation of type 2 immunity to helminths by mast cells", Gut Microbes, 2012, Vol. 3, Issue 5, Pages 476-481. Abstract; Page 478, Col. 2, Para. 3 – Page 479, Col. 2, Para. 3.	1-53
A	TSAI, H. et al. "IL-17A and Th17 Cells in Lung Inflammation: An Update on the Role of Th17 Cell Differentiation and IL-17R Signaling in Host Defense against Infection", Clinical and Developmental Immunology, 2013, Article ID 267971, 12 pages, http://dx.doi.org/10.1155/2013/267971. Whole document.	1-53

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/050772**

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WO 2015/031778 A2	05 March 2015	WO 2015031778 A2	05 Mar 2015
		TW 201514198 A	16 Apr 2015
		US 2016213767 A1	28 Jul 2016
US 2012/0064073 A1	15 March 2012	US 2012064073 A1	15 Mar 2012
		AR 064032 A1	04 Mar 2009
		AR 071829 A1	21 Jul 2010
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		AU 733222 B2	10 May 2001
		AU 1532499 A	15 Jun 1999
		AU 735081 B2	28 Jun 2001
		AU 2598697 A	22 Oct 1997
		AU 735200 B2	05 Jul 2001
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		AU 740227 B2	01 Nov 2001
		AU 3993799 A	06 Dec 1999
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		AU 741060 B2	22 Nov 2001
		AU 3757099 A	08 Nov 1999
		AU 741133 B2	22 Nov 2001
		AU 9388198 A	12 Apr 1999
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		AU 756400 B2	09 Jan 2003
		AU 9388098 A	12 Apr 1999
		AU 756449 B2	16 Jan 2003
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/050772**

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		AU 761340 B2	05 Jun 2003
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		AU 2002313838 B2	26 May 2005
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		AU 2002318894 B2	12 May 2005
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		AU 2002330015 B2	07 Feb 2008
		AU 2002330288 B2	05 May 2005
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		AU 2002367318 A1	24 Jul 2003
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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/050772**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
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		AU 2004201760 B2	19 Jul 2007
		AU 2004201769 A1	27 May 2004
		AU 2004201769 B2	19 Jul 2007
		AU 2004237842 A1	13 Jan 2005
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		AU 2004237920 A1	13 Jan 2005
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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
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		AU 2006230703 A1	09 Nov 2006
		AU 2006235436 A1	19 Oct 2006
		AU 2006235436 A2	19 Oct 2006
		AU 2006241374 A1	14 Dec 2006
		AU 2006241374 B2	22 Oct 2009
		AU 2006252241 A1	25 Jan 2007
		AU 2006252241 B2	04 Mar 2010
		AU 2007325316 A1	05 Jun 2008
		AU 2007325316 B2	09 May 2013
		AU 2008229758 A1	06 Nov 2008
		AU 2008229758 B2	26 Jul 2012
		AU 2009200399 A1	26 Feb 2009
		AU 2009200399 B2	24 May 2012
		AU 2009200401 A1	26 Feb 2009
		AU 2009200614 A1	05 Mar 2009
		AU 2009202783 A1	30 Jul 2009
		AU 2009202783 B2	03 May 2012

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
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		AU 2009213070 A1	08 Oct 2009
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		AU 2011203347 B2	05 Sep 2013
		AU 2012211366 A1	23 Aug 2012
		AU 2012216360 A1	13 Sep 2012
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		AU 2015200139 A1	05 Feb 2015
		AU 2015200139 B2	21 Jul 2016
		BR PI0510883 A	26 Dec 2007
		BR PI0515616 A	15 Jul 2008
		BR PI0609729 A2	20 Apr 2010
		BR PI0719288 A2	15 Apr 2014
		CA 2188017 A1	02 Nov 1995
		CA 2249206 A1	09 Oct 1997
		CA 2270869 A1	14 May 1998
		CA 2293740 A1	23 Dec 1998
		CA 2301847 A1	04 Mar 1999
		CA 2303834 A1	25 Mar 1999
		CA 2304102 A1	01 Apr 1999
		CA 2304129 A1	01 Apr 1999
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		CA 2305385 A1	29 Apr 1999
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