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(54) Title: COMPOUNDS AND METHODS FOR THE MODULATION OF IMMUNE RESPONSES

(57) Abstract: Methods and compositions for the modification of immune response by modulating of the Notch signaling pathway are provided, together with methods for the treatment of disorders characterized by the presence of an unwanted immune response. Such compositions comprise components derived from Mycobacteria, such as Mycobacterium vaccae.

COMPOUNDS AND METHODS FOR THE MODULATION OF IMMUNE RESPONSES

Reference to Related Applications

This application claims priority to U.S. Provisional Patent Application no. 60/308,446, filed July 26, 2001.

Technical Field

The present invention relates generally to the modification of immune system responses. In particular, the invention is related to compositions and methods for the modification of T cell responses by means of modulating the expression of molecules involved in the Notch signaling and Toll-like receptor signaling pathways, and for the treatment of disorders in which these pathways play a role.

Background of the Invention

Certain disorders, such as autoimmune disorders (for example, multiple sclerosis, rheumatoid arthritis, Type I diabetes mellitus, psoriasis, systemic lupus erythematosus and scleroderma), allergic disorders and graft rejection, are characterized by the presence of an undesirable and abnormal immune response to either a self or foreign antigen. In such disorders, suppression of the immune response, such as by induction of a negative T cell response or induction of tolerance towards the antigen, is thus highly desirable.

Recognition of an antigen by naïve CD4+ T cells in the peripheral immune system can lead to either activation of an immune response against the antigen or to the induction of tolerance wherein T cells become refractory to further stimulation with antigen. The choice between immune activation and tolerance is controlled by signals delivered by antigen presenting cells (APCs) at the time of initial presentation of the antigen by the APC. Once tolerance has been induced in a small number of T cells (known as T regulatory, or Tr cells), this tolerance can be transmitted to other T cells, thereby actively suppressing an immune

response to the antigen. This phenomenon is known as "infectious tolerance" or "linked suppression". The induction of tolerance in naïve T cells by Tr cells is believed to occur either through direct cell-cell interactions or by secretion of inhibitory cytokines, such as IL-4, IL-10 and TGF-beta.

The Notch signaling pathway is known to play an important role in regulating cell growth and differentiation. Proteins of the Notch family are large transmembrane proteins which function as receptors and that were originally identified in *Drosophila*. In mammals, four different Notch receptors (known as Notch 1-4) and at last five different ligands (Jagged-1, Jagged-2, Delta-like 1, Delta-like 3 and Delta-like 4) have been identified, with Jagged being the mammalian homologue of the Serrate ligand identified in *Drosophila*. The nucleotide sequences of the human *Notch* and *Delta* genes, and the amino acid sequences of their encoded proteins are disclosed in International Patent Publication WO 92/19734. The Notch signaling pathway is highly conserved from *D. melanogaster* through to humans, indicating the importance of this pathway in regulating cell growth anddifferentiation.

Hoyne et al. (*Immunology 100*:281-288, 2000), have demonstrated that expression of Notch ligands on T cells and APCs can lead to the development of T-cell tolerance. More specifically, Hoyne et al. propose that recognition of antigen on APCs which also express Notch ligands induces naïve T cells to differentiate into Tr cells. The activated Tr cell then expresses a Notch ligand (such as Delta) at its surface. This in turn engages Notch on neighboring naïve T cells, thereby directly influencing the growth of naïve T cells, and leading to linked suppression. Modification of the Notch signaling pathway, for example by modulation of expression of a Notch receptor or ligand, may thus be employed to modify or suppress an undesirable immune response in a disorder by inducing tolerance to a particular antigen.

Interaction of Notch with its ligands has been shown to trigger the release of the intracellular domain of Notch (N^{IC}) which in turn binds to either Deltex or CBF-1, a sequence-specific DNA transcription factor also known as RBP-Jκ. By binding to Deltex or CBF1, N^{IC} can alter the capacity of these molecules to regulate transcription of various genes. Activation of Deltex can result in repression of the basic helix-loop-helix protein E47, which is a regulator of B and T cell development and, more specifically, is involved in the

determination of B versus T cell fate. Binding of N^{IC} to CBF-1 activates transcription of the Hairy Enhancer of Split (HES) family of proteins. Disruption of HES has severe consequences on the immune system, including defects in thymic development. Specifically, HES-1 has been shown to repress CD4 expression and to affect early thymocyte precursors. Binding of N^{IC} to CBF-1 also increases expression of NF-κB2, whose activity has been associated with protection from apoptosis in lymphoid tissue (Oswald et al. *Mol. Cell. Biol. 18*:207-2088, 1998). Notch expression has been shown to rescue cells from apoptosis (Deftos et al. *Immunity 9*:777-786, 1998; Jehn et al. *J. Immunol. 162*:635-638, 1999; and Shelly et al. *J. Cell. Biochem. 73*:164-175, 1999), and it has been suggested that Notch expression may affect cell fate through direct regulation of apoptosis (Osborne et al. *Immunity 11*:653-663, 1999). More recently, the proteins Lunatic Fringe, Manic Fringe and Radical Fringe have been shown to act as potent regulators of Notch-1 expression (see, for example, Koch et al. (*Immunity 15*:225-236, 2001)). These proteins may regulate Notch-1 activation in lymphoid precursors to ensure that T and C cells develop in different tissues. Other molecules known to involved in Notch signaling include Numb, which inhibits Notch signaling; presenilin1, which is a Notch signaling regulator; HERP1 and 2, which are both downstream signaling targets; and the basic helix-loop-helix (bHLH) transcription factor HASH1 which has recently been shown to be degraded by activated Notch (Sriuranpong et al, *Mol. Cell. Biol. 22*:3129-39, 2002).

Summary of the Invention

Briefly stated, the present invention provides compositions and methods for suppression and modification of immune responses by modulating the expression of molecules involved in the Notch signaling and Toll-like receptor signaling pathways, together with compositions and methods for the treatment of disorders characterized by an unwanted immune response, such as autoimmune disorders, allergic disorders and graft rejection.

In one aspect, the present invention provides methods for modulating the expression of Notch ligands on antigen present cells, such as dendritic cells and macrophages, by contacting the antigen presenting cells with a composition described herein. In a further aspect, methods for modulating Notch and/or Toll-like receptor signaling in a population of cells, either *in vivo*

or *in vitro*, are provided, such methods comprising contacting the cells with a composition of the present invention. In yet another aspect, methods are provided for modifying an immune response to an antigen in a subject, and for stimulating infectious tolerance to an antigen in a subject, such methods comprising administering to the subject an effective amount of one or more of the compositions described herein.

In related aspects, the present invention provides methods for the treatment of a disorder characterized by an unwanted immune response in a patient, such methods comprising administering to the patient a composition of the present invention. In certain embodiments, the disorder is selected from the group consisting of autoimmune disorders (including, but not limited to, multiple sclerosis, rheumatoid arthritis, Type I diabetes mellitus, psoriasis, systemic lupus erythematosus and scleroderma), allergic diseases and graft rejection.

As discussed above, the Notch signaling pathway is also involved in apoptotic cell death mechanisms. Specifically, when Notch is expressed, cells are protected from apoptotic cell death. According to additional aspects of the present invention, methods are provided for treatment of a disorder characterized by undesired apoptotic cell death, and for treatment of a disorder characterized by undesired cell proliferation, such methods comprising modulating the Notch signaling pathway by administering a composition described herein.

In certain embodiments, the inventive methods comprise administering a composition, wherein the composition comprises inactivated mycobacterial cells or a derivative thereof, such as delipidated and deglycolipidated mycobacterial cells. In preferred embodiments, the delipidated and deglycolipidated cells are prepared from *M. vaccae*, *M. tuberculosis* or *M. smegmatis*. In further embodiments, the inventive methods comprise administering a composition comprising peptidoglycan.

In other embodiments, the compositions employed in the inventive methods comprise a derivative of delipidated and deglycolipidated mycobacterial cells, the derivative being selected from the group consisting of: delipidated and deglycolipidated mycobacterial cells that have been treated by acid hydrolysis; delipidated and deglycolipidated mycobacterial cells that have been treated by alkaline hydrolysis; delipidated and deglycolipidated mycobacterial cells that have been treated with periodic acid; delipidatedand deglycolipidated

mycobacterial cells that have been treated with Proteinase K; and delipidated and deglycolipidated mycobacterial cells that have been treated by anhydrous hydrofluoric acid hydrolysis. In specific embodiments, such derivatives are prepared from *M. vaccae*, *M. tuberculosis* or *M. smegmatis*. The derivatives of delipidated and deglycolipidated *M. vaccae* preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of delipidated and deglycolipidated *M. vaccae* contain glucosamine in an amount greater than 3.7% of total carbohydrate, preferably greater than 5% total carbohydrate and more preferably greater than 7.5% total carbohydrate.

In yet another aspect, the compositions disclosed herein comprise an isolated polypeptide derived from *Mycobacterium vaccae* or an isolated polynucleotide encoding such a polypeptide, such polypeptides comprising at least an immunogenic portion of an *M. vaccae* antigen, or a variant thereof. In specific embodiments, such polypeptides comprise an amino acid sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 27-52; (b) sequences encoded by any one of SEQ ID NO: 1-26; (c) sequences having at least about 75% identity to a sequence recited in SEQ ID NO: 27-52; (d) sequences having at least about 90% identity to a sequence recited in SEQ ID NO: 27-52, as measured using alignments produced by the computer algorithm BLASTP as described below.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Fig. 1 illustrates the re-suspension of DD-*M. vaccae* and DD-*M. vaccae*-KOH.

Fig. 2 shows the suppression by DD-*M. vaccae* (Q1) and the DD-*M. vaccae* derivatives Q2 (DD-*M. vaccae*-KOH), Q3 (DD-*M. vaccae*-acid), Q4 (DD-*M. vaccae*-periodate), Q6 (DD-*M. vaccae*-KOH-periodate), P5 (DD-*M. vaccae*-KOH-acid) and P6 (DD-*M. vaccae*-KOH-periodate) of ovalbumin-induced airway eosinophilia in mice vaccinated intranasally with these compounds. Control mice received PBS.

Fig. 3 illustrates the effect of immunization with DD-*M. vaccae* on airway eosinophilia when administered either one day prior, at the time of, or one day after challenge with OVA.

Fig. 4 shows the stimulation of IL-10 production in THP-1 cells by derivatives of DD-*M. vaccae*.

Fig. 5 illustrates the effect of immunization with DD-*M. vaccae*, DD-*M. tuberculosis* and DD-*M. smegmatis* on airway eosinophilia.

Fig. 6 illustrates TNF-α production by human PBMC and non-adherent cells stimulated with DD-*M. vaccae*.

Figs. 7A and 7B illustrate IL-10 and IFN-γ production, respectively, by human PBMC and non-adherent cells stimulated with DD-*M. vaccae*.

Figs. 8A-C illustrate the stimulation of CD69 expression on αβT cells, γδT cells and NK cells, respectively, by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 9A-D illustrate the effect of heat-killed *M. vaccae*, DD-*M. vaccae* and *M. vaccae* recombinant proteins on the production of IL-1β, TNF-α, IL-12 and IFN-γ, respectively, by human PBMC.

Figs. 10A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the production of IL-1β, TNF-α and IL-12, respectively, by human PBMC.

Figs. 11A-D illustrate the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively, in human PBMC by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 12A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the expression of CD40, CD80 and CD86, respectively, by dendritic cells.

Fig. 13 illustrates the enhancement of dendritic cell mixed lymphocyte reaction by the recombinant *M. vaccae* protein GV-23.

Fig. 14 illustrates real-time PCR analysis demonstrating that treatment of mice with AVAC produced increases in expression of Notch receptors, ligands, and downstream targets.

Fig. 15A-C illustrate the effect of heat-killed *M. vaccae*, DD-*M. vaccae* (referred to in the Figure as PVAC) and AVAC, respectively, on the expression of genes involved in Notch signaling in THP-1 cells.

Fig. 16 illustrates the effect of intranasal administration of AVAC and DD-*M. vaccae* (referred to in the Figure as PVAC) in mice on expression of genes involved in Notch signaling.

Fig. 17 illustrates the effect of intraperitoneal administration of AVAC in mice on the expression of cytokines and genes involved in Notch signaling

Fig. 18 shows the production of IL-12p40 by THP-1 cells in response to increasing concentrations of *M. vaccae* derivatives.

Fig. 19 shows the production of IL-12p40, IL-23p19 and IL-12p35 mRNA in THP-1 cells in response to AVAC, DD-*M. vaccae*, heat-killed *M. vaccae* and *M. vaccae* glycolipids.

Figs. 20A-C illustrate the production of IL-12p40 by THP-1 cells cultured with antibodies to Toll-like receptors and either heat-killed *M. vaccae*, DD-*M. vaccae* or AVAC, respectively.

Figs. 21A-C illustrate the production of TNF-alpha by THP-1 cells cultured with antibodies to Toll-like receptors and either heat-killed *M. vaccae*, DD-*M. vaccae* or LPS, respectively

Fig. 22 shows the production of IL-10 by THP-1 cells cultured with antibodies to Toll-like receptors and heat-killed *M. vaccae*

Fig. 23 illustrates the production of IL-10 by THP-1 cells cultured with MAP kinase inhibitors and AVAC.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for modulating immune responses by modification of the Notch signaling pathway. The inventive compositions and methods may thus be employed in the treatment of disorders characterized by the presence of an unwanted immune response to either a self antigen or a

foreign antigen, such as autoimmune disorders, allergic disorders and graft rejection.. Examples of autoimmune disorders include multiple sclerosis, rheumatoid arthritis, Type I diabetes mellitus, psoriasis, systemic lupus erythematosus and scleroderma. Examples of allergic disorders include atopic dermatitis, eczema, asthma, allergic rhinitis, contact allergies and hypersensitivities.

Certain pathogens, such as *M. tuberculosis*, as well as certain cancers, are effectively contained by an immune attack directed by $CD4^+$ T cells, known as cell-mediated immunity. Other pathogens, such as poliovirus, also require antibodies, produced by B cells, for containment. These different classes of immune attack (T cell or B cell) are controlled by different subpopulations of $CD4^+$ T cells, commonly referred to as Th1 and Th2 cells. The two types of Th cell subsets have been well characterized and are defined by the cytokines they release upon activation. The Th1 subset secretes IL-2, IFN-γ and tumor necrosis factor, and mediates macrophage activation and delayed-type hypersensitivity response. The Th2 subset releases IL-4, IL-5, IL-6 and IL-10, which stimulate B cell activation. The Th1 and Th2 subsets are mutually inhibiting, so that IL-4 inhibits Th1-type responses, and IFN-γ inhibits Th2-type responses.

Amplification of Th1-type immune responses is central to a reversal of disease in many disorders. IL-12 has been shown to up-regulate Th1 responses, while IL-10 has been shown to down-regulate Th2 responses. The inventors have discovered that both delipidated and deglycolipidated *M. vaccae* cells (referred to herein as DD-*M. vaccae*) and delipidated and deglycolipidated *M. vaccae* cells further treated by acid hydrolysis (referred to herein as AVAC) have pronounced immunoregulatory effects on both Th2 and Th1 cells. For example, as detailed below, the inventors have demonstrated the efficacy of both DD-*M. vaccae* and AVAC in the treatment of asthma employing a mouse model. These compositions are believed to be effective in the treatment of diseases such as asthma due to their ability to down-regulate asthma-inducing Th2 immune responses, as shown by the reduction in total IgE and antigen-specific IgE and IgG1.

In clinical trials on the effectiveness of DD-*M. vaccae* in the treatment psoriasis, local injections of DD-*M. vaccae* were observed to lead to clearance of distant skin lesions, demonstrating the involvement of a systemic mechanism of action. No *in vitro* proliferation

in response to DD-*M. vaccae* stimulation was observed in peripheral blood mononuclear cells (PBMC) taken from DD-*M. vaccae*-treated patients, thereby indicating the lack of a specific T cell response to DD-*M. vaccae*. Experimental data is presented, below, in Example 9.

As described below, DD-*M. vaccae* is ingested by cells of the THP-1 human monocytic cell line and stimulates these cells to secrete IL-10 and IL-12. DD-*M. vaccae* stimulates blood-derived human dendritic cells to upregulate the expression of CD40, CD80 and CD86 costimulatory molecules *in vitro*. T cell and NK cells show increased expression of the CD69 activation molecule when exposed to DD-*M. vaccae*, and the antigen presenting function of mouse dendritic cells is enhanced when bone marrow derived dendritic cells are pre-tested with DD-*M. vaccae in vitro*. Taken together, these results indicate that DD-*M. vaccae* modifies the response to endogenous psoriatic antigen by affecting antigen presentation.

As the clinical effects of DD-*M. vaccae* on psoriasis are systemic and distant psoriatic lesions are cleared following local injection of DD-*M. vaccae*, it is likely that DD-*M. vaccae* is transported to the lymph nodes where it influences APCs and T cells. Alternatively, either APCs or both APCs and regulatory T cells activated by DD-*M. vaccae* migrate to lymph nodes and the circulation. The APCs then terminate the generation of pathologic T cells, and T cells down regulating psoriatic pathology proliferate either in the lymph nodes or systemically.

While the expression of costimulatory molecules (CD40, CD80 and CD86) by antigen presenting cells is required for antigen presentation, and the secretion of IL-10 is likely to be important in regulating T cell responses, other molecules are required to generate T regulatory cells as a population distinct from effector T helper cells. As discussed above, the Notch ligand family of molecules is known to determine fate of cells during T cell development. Genes and molecules that determine differentiation of T cells during development are likely to influence the differentiation of T cell subsets during an immune response. The fact that DD-*M. vaccae* and its derivatives do not suppress antigen presentation and stimulate cytokine production, indicates that they may be successfully employed to modify an immune response to an antigen at the time of antigen presentation, and may also suppress an immune response that has occurred after antigen presentation.

As detailed below, the inventors have demonstrated that a derivative of DD-*M. vaccae*, namely AVAC, induces production of Notch ligands on antigen presenting cells (APCs). Recognition of an antigen on these up-regulated APCs, induces naïve T cells to differentiate into regulatory T (Tr) cells and to express a Notch ligand. The Notch ligand on the Tr cells in turn interacts with Notch on neighboring naïve T cells, leading to the induction of infectious tolerance to the antigen. The inventors have also demonstrated that AVAC, DD-*M. vaccae*, inactivated *M. vaccae* and *M. vaccae* glycolipids modulate expression of various genes involved in Notch signaling both *in vitro* and *in vivo*, as well as genes involved in Toll-like receptor and cytokine signaling.

While not wishing to be bound by theory, the inventors believe, based on the experimental results presented below, that interaction of *M. vaccae*, DD-*M. vaccae* and AVAC with human myelomonocytic THP-1 cells is mediated in part by the specific binding of *M. vaccae*-derived cell wall components, principally peptidoglycan, to the extracellular domain of Toll-like receptor 2 (TLR2), one of several pathogen receptors expressed by these cells. Ligation of TLR2 then initiates an intracellular signaling cascade leading to the transcription of cytokine genes and translation of cytokine mRNA into biologically active protein. The cytokines so elicited have a variety of biological effects, including the capacity to influence expression of: genes involved in Notch signaling; TLR signaling genes themselves; and other inflammation-associated genes such as that for the calcium-binding protein MRP8.

As described in detail below, the inventors have demonstrated that *M. vaccae* derivatives up- or down-regulate expression of genes encoding Notch receptors, Notch ligands, downstream targets of Notch signaling, and Notch-active glycosyltransferases in human THP-1 cells. It is believed that this occurs partly via the actions of cytokines and cytokine signaling pathway mediators induced by Toll-like receptor (TLR) signaling, and partly via *bona fide* Notch signaling. As discussed above, Notch signaling occurs in cells expressing Notch receptors, and is initiated when Notch receptors are specifically ligated by Notch ligands. Although THP-1 cells express all of the Notch receptors and ligands described herein, it is likely that very little Notch signaling occurs in cultures of free-floating THP-1 cells in the absence of external stimuli. However, by ligating TLR2 on adjacent THP-1 cells,

inactivated *M. vaccae*, DD-*M. vaccae* and AVAC bring THP-1 cells into very close contact with one another, thereby facilitating multiple productive interactions between Notch receptors and Notch ligands, which in turn leads to signal transduction in the Notch-bearing cell. Ligation of Notch receptor leads to proteolytic release of Notch intracellular domain (N^{IC}), the intracellular mediator responsible for entering the nucleus and, in co-operation with additional molecules, initiating transcription of: downstream Notch signaling genes such as HES1, Deltex and HERP; Notch receptor, Notch ligand, and Notch-active glycosyltransferase genes by one or more autocrine feedback loops; and other genes whose expression is influenced by Notch signaling (for example, Numb). Within this framework, recognition of *M. vaccae* derivatives by THP-1 cells is mediated by TLR2, and decision-making is mediated by both downstream products of TLR signaling (changes in expression of TLR and cytokine genes) and by Notch signaling.

As used herein the term "inactivated *M. vaccae*" refers to *M. vaccae* cells that have either been killed by means of heat, as detailed below in Example 1, or by exposure to radiation, such as ^{60}Cobalt at a dose of 2.5 megarads, or by any other inactivation technique. As used herein, the term "modified *M. vaccae*" includes delipidated *M. vaccae* cells, deglycolipidated *M. vaccae* cells, *M. vaccae* cells that have been both delipidated and deglycolipidated (DD-*M. vaccae*), and derivatives of delipidated and deglycolipidated *M. vaccae* cells. DD-*M. vaccae* may be prepared as described below in Example 1, with the preparation of derivatives of DD-*M. vaccae* being detailed below in Example 2. The preparation of delipidated and deglycolipidated *M. tuberculosis* (DD-*M. tuberculosis*) and *M. smegmatis* (DD-*M. smegmatis*) is described in Example 5, below. Derivatives of DD-*M. tuberculosis* and DD-*M. smegmatis*, such as acid-treated, alkali-treated, periodate-treated, proteinase K-treated, and/or hydrofluoric acid-treated derivatives, may be prepared using the procedures disclosed herein for the preparation of derivatives of DD-*M. vaccae*.

The derivatives of DD-*M. vaccae* preferably contain galactose in an amount less than 9.7% of total carbohydrate, more preferably less than 5% of total carbohydrate, and most preferably less than 3.5% total carbohydrate. In certain embodiments, the derivatives of DD-*M. vaccae* preferably contain glucosamine in an amount greater than 3.7% of total carbohydrate, more preferably greater than 5% total carbohydrate, and most preferably greater

than 7.5% total carbohydrate. Derivatives prepared by treatment of DD-*M. vaccae* with alkali, such as DD-*M. vaccae*-KOH (also known as KVAC), have a reduced number of ester bonds linking mycolic acids to the arabinogalactan of the cell wall compared to DD-*M. vaccae*, and are thus depleted of mycolic acids. Derivatives prepared by treatment with acid, such as DD-*M. vaccae*-acid (also referred to as AVAC), have a reduced number of phosphodiester bonds attaching arabinogalactan sidechains to the peptidoglycan of the cell wall, and are therefore depleted of arabinogalactan. In addition, such derivatives are depleted of DNA. Derivatives prepared by treatment of *DD-M. vaccae* with periodate, such as DD-*M. vaccae*-periodate (also known as IVAC), have a reduced number of cis-diol-containing sugar residues compared to DD-*M. vaccae* and are depleted of arabinogalactan. Derivatives prepared by treatment of *DD-M. vaccae* with Proteinase K (such as the derivative referred to as EVAC) are depleted of proteins and peptides. Derivatives prepared by treatment with hydrofluoric acid, such as *DD-M. vaccae*-KOH treated with hydrofluoric acid (referred to as HVAC), are depleted of glycosidic bonds.

In certain embodiments, compositions that may be effectively employed in the inventive methods include polypeptides that comprise at least a functional portion of an *M. vaccae* antigen, or a variant thereof. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising a functional portion of an antigen may consist entirely of the functional portion, or may contain additional sequences. The additional sequences may be derived from the native *M. vaccae* antigen or may be heterologous.

A "functional portion" as used herein means a portion of an antigen that possesses an ability to modulate the expression of a protein involved in the Notch signaling pathway. The ability of an antigen, or a portion thereof, to modulate expression of a protein involved in the Notch signaling pathway may be determined as described below in Examples 11-14.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially

synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments. Antisense polynucleotides and techniques involving antisense polynucleotides are well known in the art and are described, for example, in Robinson-Benion *et al.*, "Antisense techniques," *Methods in Enzymol.* 254(23):363-375, 1995; and Kawasaki *et al.*, *Artific. Organs* 20 (8):836-848, 1996.

As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants, and include polynucleotides that encode identical amino acid sequences or essentially identical sequences differing by codon alterations that reflect the degeneracy of the genetic code. In addition to these "silent variations", it is understood by those skilled in the art that conservative substitutions can be made by substituting particular amino acids with chemically similar amino acids without changing the function of the polypeptide (see e.g., Creighton, "Proteins", W.H. Freeman and Company (1984).

Variant sequences (polynucleotide or polypeptide) preferably exhibit at least 75%, more preferably at least 90%, and most preferably at least 95% identity to a sequence of the present invention. The percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive (queried) sequence, and multiplying the result by 100. By way of example only, assume a queried polynucleotide having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the default parameters as described below. The 23 nucleotide hit includes 21 identical nucleotides, one gap and one different nucleotide. The

percentage identity of the queried polynucleotide to the hit in the EMBL database is thus 21/220 times 100, or 9.5%. The percentage identity of polypeptide sequences may be determined in a similar fashion.

Polynucleotide and polypeptide sequences may be aligned, and percentages of identical residues in a specified region may be determined against another polynucleotide or polypeptide sequence, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. Polynucleotides may also be analyzed using the BLASTX algorithm, which compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. The percentage identity of polypeptide sequences may be examined using the BLASTP algorithm. The BLASTN, BLASTP and BLASTX algorithms are available on the NCBI anonymous FTP server under /blast/executables/ and are available from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894, USA. The BLASTN algorithm Version 2.0.11 [Jan-20-2000], set to the parameters described below, is preferred for use in the determination of polynucleotide variants according to the present invention. The BLASTP algorithm, set to the parameters described below, is preferred for use in the determination of polypeptide variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, BLASTP and BLASTX, is described in the publication of Altschul, *et al.*, *Nucleic Acids Res. 25*:3389-3402, 1997.

The FASTA and FASTX algorithms are available on the Internet, and from the University of Virginia by contacting the Vice Provost for Research, University of Virginia, P.O. Box 9025, Charlottesville, VA 22906-9025, USA. The FASTA algorithm, set to the default parameters described in the documentation and distributed with the algorithm, may be used in the determination of polynucleotide variants. The readme files for FASTA and FASTX Version 1.0x that are distributed with the algorithms describe the use of the algorithms and describe the default parameters. The use of the FASTA and FASTX algorithms is described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-2448, 1988; and Pearson, *Methods in Enzymol.* 183:63-98, 1990.

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity for polynucleotides: Unix running command with the following default parameters: blastall -p blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq –o results; and parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (blastn only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; -o BLAST report Output File [File Out] Optional.

The following running parameters are preferred for determination of alignments and similarities using BLASTP that contribute to the E values and percentage identity of polypeptide sequences: blastall –p blastp –d swissprotdb –e 10 -G 0 -E 0 –v 30 –b 30 –i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence. The BLASTN, FASTA and BLASTP algorithms also produce "Expect" values for polynucleotide and polypeptide alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the

sequences then have a probability of 90% of being related. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN algorithm. E values for polypeptide sequences may be determined in a similar fashion using various polypeptide databases, such as the SwissProt database.

According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences having the same number or fewer nucleic or amino acids than each of the polynucleotides or polypeptides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99% probability of being the same as the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN, FASTA or BLASTP algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN algorithm set at the default parameters. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer amino acids than a polypeptide of the present invention that has at least a 99% probability of being the same as the polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the default parameters.

In addition to having a specified percentage identity to an inventive polynucleotide or polypeptide sequence, variant polynucleotides and polypeptides preferably have additional structure and/or functional features in common with the inventive polynucleotide or polypeptide. Polypeptides having a specified degree of identity to a polypeptide of the present invention share a high degree of similarity in their primary structure and have substantially similar functional properties. In addition to sharing a high degree of similarity in their primary structure to polynucleotides of the present invention, polynucleotides having a specified degree of identity to, or capable of hybridizing to, an inventive polynucleotide

preferably have at least one of the following features: (i) they contain an open reading frame or partial open reading frame encoding a polypeptide having substantially the same functional properties as the polypeptide encoded by the inventive polynucleotide; or (ii) they contain identifiable domains in common.

In certain embodiments, variant polynucleotides hybridize to a polynucleotide of the present invention under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the discrepancy of the genetic code, encode a polypeptide having similar enzymatic activity as a polypeptide encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NOS: 1-26 (or complements, reverse sequences, or reverse complements of those sequences) as a result of conservative substitutions are encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the inventive polynucleotide sequences or complements, reverse complements, or reverse sequences as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the inventive polypeptide sequences as a result of amino acid substitutions, insertions, and/or deletions totalling less than 10% of the total sequence length are contemplated by and encompassed within the present invention, provided the variant polypeptide has similar activity to the inventive polypeptide.

A polypeptide described herein may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In general, *M. vaccae* antigens, and polynucleotides encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from *M. vaccae* culture filtrate. Antigens may also be produced recombinantly by inserting a DNA sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, mycobacteria, insect, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

Polynucleotides encoding *M. vaccae* antigens may be obtained by screening an appropriate *M. vaccae* cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from amino acid sequences of isolated antigens. Suitable degenerate oligonucleotides may be designed and synthesized, and the screen may be performed as described, for example in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. Polymerase chain reaction (PCR) may be employed to isolate a nucleic acid probe from genomic DNA, or a cDNA or genomic DNA library. The library screen may then be performed using the isolated probe. DNA molecules encoding *M. vaccae* antigens may also be isolated by screening an appropriate *M. vaccae* expression library with anti-sera (*e.g.*, rabbit or monkey) raised specifically against *M. vaccae* antigens.

Regardless of the method of preparation, the antigens described herein have the ability to modify an immune response. More specifically, the antigens have the ability to effect the Notch signaling pathway by modulation of the expression of proteins involved in the Notch signaling pathway including, but not limited to, Notch or Notch ligands on APCs and/or T cells. The ability of an antigen to modulate the expression of proteins involved in the Notch signaling pathway may be determined as described below in Example 11-14.

Portions and other variants of *M. vaccae* antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native antigen may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

In general, regardless of the method of preparation, the polypeptides and polynucleotides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides and polynucleotides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Alternatively, a composition of the present invention may contain DNA encoding one or more polypeptides as described above, such that the polypeptide is generated *in situ*. In such compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science 259*:1745-1749, 1993 and reviewed by

Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

As noted above, the compositions describe herein may be employed for the treatment of disorders including autoimmune disorders, allergic disorders and graft rejection. When used in such methods, the compositions described herein may be administered by injection (*e.g.*, intradermal, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration), orally or epicutaneously (applied topically onto skin). In one embodiment, the compositions are in a form suitable for delivery to the mucosal surfaces of the airways leading to or within the lungs. For example, the composition may be suspended in a liquid formulation for deliveryto a patient in an aerosol form or by means of a nebulizer device.

For use in therapeutic methods, the compositions described herein may additionally contain a physiologically acceptable carrier. While any suitable carrier known to those of ordinary skill in the art may be employed in the compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed.

The preferred frequency of administration and effective dosage will vary from one individual to another. For both DD-*M. vaccae* and derivatives of DD-*M. vaccae*, the amount present in a dose preferably ranges from about 10 μg to about 1000 μg, more preferably from about 10 μg to about 100 μg. The number of doses may range from 1 to about 10 administered over a period of up to 12 months. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 5 ml.

The word "about," when used in this application with reference to the amount of active component in a dose, contemplates a variance of up to 5% from the stated amount.

The following examples are offered by way of illustration and are not limiting.

EXAMPLE 1

PREPARATION OF DELIPIDATED AND DEGLYCOLIPIDATED *M. VACCAE* CELLS (DD-*M. VACCAE*)

This example illustrates the processing of different constituents of *M. vaccae* and their immune modulating properties.

Heat-killed *M. vaccae* and *M. vaccae* culture filtrate

M. vaccae (American Type Culture Collection Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose 1 g/l) at 37°C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI) with glucose at 37°C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120°C. The culture filtrate was passaged through a 0.45 μm filter into sterile bottles.

Preparation of Delipidated and Deglycolipidated *M. vaccae* (DD-*M. vaccae*) and Compositional Analysis

To prepare delipidated *M. vaccae*, the autoclaved *M. vaccae* was pelleted by centrifugation, the pellet washed with water and collected again by centrifugation, and freeze-dried. An aliquot of this freeze-dried *M. vaccae* was set aside and referred to as lyophilised *M. vaccae*. When used in experiments it was resuspended in PBS to the desired concentration. Freeze-dried *M. vaccae* was treated with chloroform/methanol (2:1) for 60 min at room temperature to extract lipids, and the extraction was repeated once. The delipidated residue from the chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of *M. vaccae* glycolipids. The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of delipidated and deglycolipidated *M. vaccae* prepared was equivalent to 11.1% of

the starting wet weight of *M. vaccae* used. For bioassay, the delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*), was resuspended in phosphate-buffered saline by sonication, and sterilized by autoclaving.

The compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae* are presented in Table 1. Major changes are seen in the fatty acid composition and amino acid composition of DD-*M. vaccae* as compared to the insoluble fraction of heat-killed *M. vaccae*. The data presented in Table 1 show that the insoluble fraction of heat-killed *M. vaccae* contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-*M. vaccae* contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

TABLE 1

Compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae*

MONOSACCHARIDE COMPOSITION

sugar alditol	***M. vaccae***	**DD-*M. vaccae***
Inositol	3.2%	1.7%
sugar alditol	***M. vaccae***	**DD-*M. vaccae***
Ribitol *	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%

FATTY ACID COMPOSITION

Fatty acid	***M. vaccae***	**DD-*M. vaccae***
C14:0	3.9%	10.0%
C16:0	21.1%	7.3%
C16:1	14.0%	3.3%
C18:0	4.0%	1.5%
C18:1*	1.2%	2.7%
C18:1w9	20.6%	3.1%
C18:1w7	12.5%	5.9%
C22:0	12.1%	43.0%
C24:1*	6.5%	22.9%

The insoluble fraction of heat-killed *M. vaccae* contains 10% w/w of lipid, and DD-*M. vaccae* contains 1.3% w/w of lipid.

AMINO ACID COMPOSITION

nmoles/mg	*M. vaccae*	DD-*M. vaccae*
ASP	231	361
THR	170	266
SER	131	199
GLU	319	505
PRO	216	262
GLY	263	404
ALA	416	621
CYS*	24	26
VAL	172	272
MET*	72	94
ILE	104	171
LEU	209	340
TYR	39	75
PHE	76	132
GlcNH2	5	6
HIS	44	77
LYS	108	167
ARG	147	272

The total amino acid content of the insoluble fraction of heat-killed *M. vaccae* is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-*M. vaccae* is 4250 nmoles/mg, or approximately 51% w/w.

***M. vaccae* glycolipids**

The pooled 50% ethanol extracts described above were dried by rotary evaporation, redissolved in water, and freeze-dried. The amount of glycolipid recovered was 1.2% of the starting wet weight of *M. vaccae* used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

EXAMPLE 2

PREPARATION AND CHARACTERIZATION OF ADDITIONAL DERIVATIVES OF *M. VACCAE*

Alkaline hydrolysis of DD-*M. vaccae*

This procedure is intended to cleave linkages that are labile to alkaline lysis, such as the ester bonds linking mycolic acids to the arabinogalactan of the mycobacterial cell wall.

One gram of DD-*M. vaccae*, prepared as described in Example 1, was suspended in 20 ml of a 0.5% solution of potassium hydroxide (KOH) in ethanol. Other alkaline agents and solvents are well known in the art and may be used in the place of KOH and ethanol. The mixture was incubated at 37°C with intermittent mixing for 48 hours. The solid residue was harvested by centrifugation, and washed twice with ethanol and once with diethyl ether. The product was air-dried overnight. The yield was 1.01 g (101%) of KOH-treated DD-*M. vaccae*, subsequently referred to as DD-*M. vaccae*-KOH (also known as KVAC). This derivative was found to be more soluble than the other derivatives of DD-*M. vaccae* disclosed herein.

Acid hydrolysis of DD-*M. vaccae*

This procedure is intended to cleave acid-labile linkages, such as the phosphodiester bonds attaching the arabinogalactan sidechains to the peptidoglycan of the mycobacterial cell wall.

DD-*M. vaccae* or DD-*M. vaccae*-KOH (100 mg) was washed twice in 1 ml of 50 mM H_2SO_4 followed by resuspension and centrifugation. Other acids are well known in the art and may be used in place of sulphuric acid. For the acid hydrolysis step, the solid residue was resuspended in 1 ml of 50 mM H_2SO_4, and incubated at 60°C for 72 hours. Following recovery of the solid residue by centrifugation, the acid was removed by washing the residue five times with water. The freeze-dried solid residue yielded 58.2 mg acid-treated DD-*M. vaccae* (DD-*M. vaccae*-acid; also known as AVAC) or 36.7 mg acid-treated DD-*M. vaccae*-KOH (DD-*M. vaccae*-KOH-acid).

Periodic acid cleavage of DD-*M. vaccae*

This procedure is intended to cleave *cis*-diol-containing sugar residues in DD-

M. vaccae, such as the rhamnose residue near the attachment site of the arabinogalactan chains to the peptidoglycan backbone.

DD-*M. vaccae* or DD-*M. vaccae*-KOH (100 mg) was suspended in 1 ml of a solution of 1% periodic acid in 3% acetic acid, incubated for 1 hour at room temperature and the solid residue recovered by centrifugation. This periodic acid treatment was repeated three times. The solid residue was recovered by centrifugation, and incubated with 5 ml of 0.1 M sodium borohydride for one hour at room temperature. The resulting solid residue was recovered by centrifugation and the sodium borohydride treatment repeated. After centrifugation, the solid residue was washed four times with water and freeze-dried to give a yield of 62.8 mg DD-*M. vaccae*-periodate (also known as IVAC) or 61.0 mg DD-*M. vaccae*-KOH-periodate.

Resuspension of DD-*M. vaccae* and DD-*M. vaccae*-KOH

DD-*M. vaccae* and DD-*M. vaccae*-KOH (11 mg each) were suspended in phosphate-buffered saline (5.5 ml). Samples were sonicated with a Virtis probe sonicator for various times at room temperature (mini-probe, 15% output). Samples were then vortexed for sixty seconds and allowed to stand for five minutes to allow the sedimentation of large particles. The absorbance of the remaining suspension at 600 nm was measured. As shown in Fig. **1**, DD-*M. vaccae*-KOH (referred to in Fig. **1** as DDMV-KOH) was fully resuspended after one minute's sonication, and further sonication produced no further increase in the absorbance. After five minutes sonication, the resuspension of DD-*M. vaccae* (referred to in Fig. **1** as DDMV) was still incomplete as estimated from the absorbance of the suspension. These results indicate that DD-*M. vaccae*-KOH is considerably more soluble than DD-*M. vaccae*.

Proteinase K hydrolysis of DD-*M. vaccae*

This procedure is intended to digest proteins and peptides, while leaving most other materials intact.

One hundred milligrams of DD-*M. vaccae*, prepared as described in Example 1, was suspended in 9 ml water with sonication. Sodium dodecyl sulfate (SDS) was added to a final concentration of 1% w/v, and Proteinase K to a final concentration of 100 µg/ml w/v. The reaction mixture was incubated at 50°C for 16 hours. The product was harvested by

centrifugation, washed with phosphate-buffered saline and water, and lyophilized. The yield was 59 mg (59%) of Proteinase K-treated DD-*M. vaccae*, subsequently referred to as EVAC.

Hydrofluoric acid hydrolysis of KOH-treated DD-*M. vaccae*

This procedure is intended to cleave linkages that are labile to hydrolysis with anhydrous hydrofluoric acid, such as glycosidic bonds, while leaving most proteins intact.

One gram of DD-*M. vaccae*-KOH, prepared as described above, was suspended in 15 ml liquid hydrogen fluoride containing anisole as a free-radical scavenger. The mixture was incubated at 0°C with mixing for one hour. The hydrogen fluoride (HF) was removed by distillation, and the solid residue was washed with diethyl ether to remove the anisole. The resulting product was extracted with water to yield water-soluble and water-insoluble fractions. The yield was 250 mg (25%) of water-soluble material, and 550 mg (55%) of water-insoluble HF-hydrolyzed KOH-treated DD-*M. vaccae*, subsequently referred to as HVAC.

Carbohydrate compositional analysis of DD-*M. vaccae* and DD-M. *vaccae* derivatives

The carbohydrate composition of DD-*M. vaccae* and DD-*M. vaccae* derivatives was determined using standard techniques. The results are shown in Table 2, wherein DDMV represents DD-*M. vaccae*; DDMV-KOH represents DD-*M. vaccae*-KOH; DDMV-A represents DD-*M. vaccae*-acid; DDMV-I represents DD-*M. vaccae*-periodate; DDMV-KOH-A represents DD-*M. vaccae*-KOH-acid; and DDMV-KOH-I represents DD-*M. vaccae*-KOH-periodate.

TABLE 2

Carbohydrate Compositional Analysis of DD-*M. vaccae* and DD-*M. vaccae* Derivatives

Carbohydrate	DDMV	DDMV-KOH	DDMV-A	DDMV-I	DDMV-KOH-A	DDMV-KOH-I
Galactosamine	26.6*	29.2	14.9	37.7	0.3	3.9
Glucosamine	3.7	3.6	8.7	35.6	12.2	63.2
Galactose	9.7	9.2	0.7	3.4	0.0	0.0
Glucose	56.9	54.8	71.1	23.0	87.5	27.5
Mannose	3.2	3.2	4.7	0.4	0.02	5.5

Carbohydrate	DDMV	DDMV-KOH	DDMV-A	DDMV-I	DDMV-KOH-A	DDMV-KOH-I
Fucose	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

* All values in %of total carbohydrate

The results demonstrate that each of the DD-*M. vaccae* derivatives had a different carbohydrate content, as expected from the different effects of the acid, periodate or alkali treatment of the cells. In addition, DD-*M. vaccae* had a marked different carbohydrate composition when compared with the DD-*M. vaccae* derivatives. As expected, the amount of galactose in the DD-*M. vaccae*-acid and DD-*M. vaccae*-periodate derivatives was lower than in DD-*M. vaccae* and DD-*M. vaccae*-KOH. These values reflect the action of the acid and periodate in the preparation of the derivatives, cleaving the arabinogalactan sidechains from the peptidoglycan backbone.

Nucleic acid analysis of DD-*M. vaccae* and DD-*M.vaccae* derivatives

Analysis by gel electrophoresis of the nucleic acid content of DD-*M. vaccae* and the DD-*M. vaccae* derivatives after treatment with Proteinase K showed that DD-*M. vaccae*, DD-*M. vaccae*-periodate and DD-*M. vaccae*-KOH contained small amounts of DNA while no detectable nucleic acid was observed for DD-*M. vaccae*-acid.

EXAMPLE 3

EFFECT OF IMMUNIZATION WITH DD-*M. VACCAE* AND DERIVATIVES OF DD-*M. VACCAE* ON ASTHMA IN MICE

The ability of DD-*M. vaccae* and derivatives of DD-*M. vaccae* to inhibit the development of allergic immune responses was examined in a mouse model of the asthma-like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate in the airways.

BALB/cByJ mice were given 2 µg ovalbumin in 2 mg alum adjuvant by the intraperitoneal route at time 0 and 14 days, and subsequently given 100 µg ovalbumin in 50 µl phosphate buffered saline (PBS) by the intranasal route on day 28. The mice accumulated

eosinophils in their airways as detected by washing the airways of the anesthetized mice with saline, collecting the washings (broncheolar lavage or BAL), and counting the numbers of eosinophils.

DD-*M. vaccae* derivatives were prepared as described above. Groups of 10 mice were administered 200 µg of PBS, DD-*M. vaccae* or one of the DD-*M. vaccae* derivatives (Q1: DD-*M. vaccae*; Q2: DD-*M. vaccae*-KOH; Q3: DD-*M. vaccae*-acid; Q4: *M. vaccae*-periodate; Q6 and P6: DD-*M. vaccae*-KOH-periodate; P5: DD-*M. vaccae*-KOH-acid) intranasally one week before intranasal challenge with ovalbumin. As shown in Fig. **2**, statistically significant reductions were observed in the percentage of eosinophils in BAL cells collected six days after challenge with ovalbumin, compared to control mice. Furthermore, the data shows that suppression of airway eosinophilia with DD-*M. vaccae*-acid and DD-*M. vaccae*-KOH-periodate (Q3, Q6 and P6) was greater than that obtained with DD-*M. vaccae* (Q1). Control mice were given intranasal PBS. The data in Fig. **2** shows the mean and SEM per group of mice.

Eosinophils are blood cells that are prominent in the airways in allergic asthma. The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic asthma. The data shown in Fig. **2** indicate that treatment with DD-*M. vaccae* or derivatives of DD-*M. vaccae* reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-*M. vaccae* or derivatives of DD-*M. vaccae* may therefore reduce the severity of asthma and diseases that involve similar immune abnormalities, such as allergic rhinitis, atopic dermatitis and eczema.

In addition, serum samples were collected from mice immunized with either heat-killed *M. vaccae* or DD-*M. vaccae* and the level of antibodies to ovalbumin was measured by standard enzyme-linked immunoassay (EIA). As shown in Table 3 below, sera from mice infected with BCG had higher levels of ovalbumin-specific IgG1 than sera from PBS controls. In contrast, mice immunized with heat-killed *M. vaccae* or DD-*M. vaccae* had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of DD-*M. vaccae* on the asthma-inducing Th2 immune responses.

TABLE 3

Low Antigen-Specific IgG1 Serum Levels

in Mice Immunized with Heat-killed *M. vaccae* or DD-*M. vaccae*

Treatment Group	Serum IgG1	
	Mean	SEM
M .vaccae i.n.	185.00	8.3
M. vaccae s.c.	113.64	8.0
DD-*M. vaccae* i.n.	96.00	8.1
DD-*M. vaccae* s.c.	110.00	4.1
Treatment Group	Serum IgG1	
	Mean	SEM
BCG, Pasteur	337.00	27.2
BCG, Connaught	248.00	46.1
PBS	177.14	11.4

In further studies, the effects of DD-*M. vaccae*-acid (AVAC) on eosinophilia in the mouse model when administered either one day before challenge with OVA, at the time of challenge or one day after challenge were examined. As shown in Fig. **3**, suppression of eosinophilia was greatest when AVAC was administered one day before challenge or at the same time.

EXAMPLE 4

EFFECT OF DD-M. *VACCAE* DERIVATIVES ON

IL-10 PRODUCTION IN THP-1 CELLS

IL-10 has been shown to inhibit the cytokine production of Th1 cells and play a key role in the suppression of experimentally-induced inflammatory responses in skin (Berg *et al.*, *J. Exp. Med.* 182:99-108, 1995). More recently, IL-10 has been used successfully in two clinical trials to treat psoriatic patients (Reich *et al.*, *J. Invest. Dermatol.* 111:1235-1236, 1998

and Asadullah *et al.*, *J. Clin. Invest.* 101:783-794, 1998). The levels of IL-10 produced by a human monocytic cell line (THP-1) cultured in the presence of derivatives of DD-*M. vaccae* were assessed as follows.

THP-1 cells (ATCC Number TIB-202) were cultured in RPMI medium (Gibco BRL Life Technologies) supplemented with 0.5 mg/l streptomycin, 500 U/l penicillin, 2 mg/l L-glutamine, $5x10^{-5}$ M β-mercaptoethanol and 5% fetal bovine serum (FBS). One day prior to the assay, the cells were subcultured in fresh media at 5×10^5 cells/ml. Cells were incubated at 37 °C in humidified air containing 5% CO_2 for 24 hours and then aspirated and washed by centrifugation with 50 ml of media. The cells were resuspended in 5 ml of media and the cell concentration and viability determined by staining with Trypan blue (Sigma, St Louis MI) and analysis under a hemocytometer. DD-*M. vaccae* derivatives (prepared as described above) in 50 µl PBS and control stimulants were added in triplicate to wells of a 96 well plate containing 100 µl of medium and appropriate dilutions were prepared. Lipopolysaccharide (LPS) (300µg/ml; Sigma) and PBS were used as controls. To each well, 100 µl of cells were added at a concentration of 2×10^6 cells/ml and the plates incubated at 37 °C in humidified air containing 5% CO_2 for 24 hours. The level of IL-10 in each well was determined using human IL-10 ELISA reagents (PharMingen, San Diego CA) according to the manufacturer's protocol. As shown in Fig. **4**, the acid and periodate derivatives of DD-*M. vaccae* were found to stimulate significant levels of IL-10 production. The PBS control, DD-*M. vaccae*-KOH, DD-*M. vaccae*-KOH-periodate, and DD-*M. vaccae*-KOH-acid derivatives did not stimulate THP-1 cells to produce IL-10.

EXAMPLE 5

PREPARATION AND COMPOSITIONAL ANALYSIS OF DELIPIDATED AND DEGLYCOLIPIDATED *M. TUBERCULOSIS* (DD-*M. TUBERCULOSIS*) AND *M. SMEGMATIS* (DD-*M. SMEGMATIS*)

***M. tuberculosis* and *M. smegmatis* culture filtrate**

Cultures of *Mycobacterium smegmatis* (*M. smegmatis*, ATCC Number 27199) were grown as described in Example 1 for *M. vaccae* in Medium 90 with 1% added glucose. After incubation at 37°C for 5 days, the cells were harvested by centrifugation and the culture

filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. smegmatis* organisms per ml. The cell suspension was then autoclaved for 15 min at 120°C. The culture filtrate was passaged through a 0.45 μm filter into sterile bottles.

Cultures of *M. tuberculosis* strain H37Rv (ATCC Number 27294) were grown at 37°C in GAS medium (0.3 g Bactocasitone (Difco Laboratories, Detroit MI), 0.05 g ferric ammonium citrate, 4 g K_2HPO_4, 2 g citric acid, 1 g L-alanine, 1.2 g $MgCl_2.6H_2O$, 0.6 g K_2SO_4, 2 g NH_4Cl, 1.8 ml NaOH (10 N), 5 ml glycerol, pH 7.0) for five days. Harvesting and further treatment of cells are as described above for *M. smegmatis* cells.

Preparation of Delipidated and Deglycolipidated *M. tuberculosis* (DD-*M. tuberculosis*) and Delipidated and Deglycolipidated *M. smegmatis* (DD-*M. smegmatis*) and Compositional Analysis.

To prepare delipidated and deglycolipidated *M. tuberculosis* (DD-*M. tuberculosis*) and *M. smegmatis* (DD-*M. smegmatis*), autoclaved *M. tuberculosis* and *M. smegmatis* were pelleted by centrifugation, the pellet washed with water and collected again by centrifugation, and freeze-dried. An aliquot of this freeze-dried *M. tuberculosis* and *M. smegmatis* was set aside and referred to as lyophilized *M. tuberculosis* and *M. smegmatis*, respectively. When used in experiments, the lyophilized material was resuspended in PBS to the desired concentration.

Delipidated and deglycolipidated *M. tuberculosis* (DD-*M. tuberculosis)* and *M. smegmatis* (DD-*M. smegmatis*) were prepared as described in Example 1 for the preparation of DD-*M. vaccae*. For bioassay, the freeze-dried DD-*M. tuberculosis* and DD-*M. smegmatis* were resuspended in phosphate-buffered saline (PBS) by sonication, and sterilized by autoclaving.

The compositional analyses of DD-*M. tuberculosis* and DD-*M. smegmatis* are presented in Table 4 and Table 5. Major differences are seen in some components of the monosaccharide composition of DD-*M. tuberculosis* and DD-*M. smegmatis* compared with the monosaccharide composition of DD-*M. vaccae*. The data presented in Table 4 show that

DD-*M. tuberculosis* and DD-*M. smegmatis* contain 1.3% and 0.0 mol% glucose, respectively, compared with 28.1 mol% for DD-*M. vaccae*.

The amino acid composition of DD-*M. tuberculosis* and DD-*M. smegmatis* is presented in Table 5. DD-*M. tuberculosis* contains 6537.9 nmoles/mg amino acids, or approximately 78.5% w/w, and DD-*M. smegmatis* contains 6007.7 nmoles/mg amino acids, which is approximately 72.1% w/w protein. When compared with the amino acid analysis of DD-*M. vaccae*, DD-*M. tuberculosis* and DD-*M. smegmatis* contain more total % protein than DD-*M. vaccae* (55.1 %).

TABLE 4

Monosaccharide Composition of DD-*M. tuberculosis* and DD-*M. smegmatis*

Monosaccharide	*M. tuberculosis*		*M. smegmatis*	
	wt%	mol%	wt%	mol%
Inositol	0.0	0.0	0.0	0.0
Glycerol	9.5	9.7	15.2	15.5
Arabinose	69.3	71.4	69.3	70.0
Xylose	ND*	ND	3.9	4.0
Mannose	3.5	3.0	2.2	1.9
Glucose	1.5	1.3	0.0	0.0
Galactose	12.4	10.7	9.4	8.0

* Not done

TABLE 5

Amino Acid Composition of DD-*M. tuberculosis* and DD-*M. smegmatis*

Amino acid	*M. tuberculosis*		*M. smegmatis*	
	Total Protein nmoles/mg	Total % protein	Total Protein nmoles/mg	Total % protein
ASP	592.5	9.1	557.0	9.3
THR	348.1	5.3	300.5	5.0
SER	218.6	3.3	252.6	4.2
GLU	815.7	12.5	664.9	11.1
PRO	342.0	5.2	451.9	7.5
GLY	642.9	9.8	564.7	9.4

ALA	927.9	14.2	875.1	14.6
CYS	31.8	0.5	20.9	0.3
VAL	509.7	7.8	434.8	7.2
MET	122.6	1.9	113.1	1.9
ILE	309.9	4.7	243.5	4.1
LEU	542.5	8.3	490.8	8.2
TYR	116.0	1.8	108.3	1.8
PHE	198.9	3.0	193.3	3.2
HIS	126.1	1.9	117.2	2.0
LYS	272.1	4.2	247.8	4.1
ARG	421.0	6.4	371.7	6.2

EXAMPLE 6

EFFECT OF IMMUNIZATION WITH DD-*M. TUBERCULOSIS* AND DD-*M. SMEGMATIS* ON ASTHMA IN MICE

The ability of DD-*M. tuberculosis* and DD-*M. smegmatis* to inhibit the development of allergic immune responses was examined in a mouse model of the asthma-like allergen-specific lung disease, as described above in Example 3. The results illustrate the effect of immunization with DD-*M. tuberculosis* and DD-*M. smegmatis* on the suppression of eosinophilia in the airways, illustrating their immune modulating properties.

BALB/cByJ female mice were sensitized to OVA by intraperitoneal injection of 200 µl of an emulsion containing 10 µg OVA and 1 mg Alum adjuvant on days 0 and 7. On days 14 and 21, mice were anesthetized and vaccinated intranasally or intradermally with 200 µg of DD-*M. vaccae*, DD-*M. tuberculosis*, DD-*M. smegmatis* or PBS. On days 28 and 32, mice were anesthetized and challenged intranasally with 100 µg OVA. Mice were sacrificed on day 35 and bronchoalveolar lavage (BAL) performed using PBS. BAL cell samples were analyzed by flow cytometry to determine the eosinophil content (% eosinophils). Total BAL eosinophil numbers were obtained by multiplying the percentage eosinophil value by the total number of leukocytes obtained, with the latter value being determined using a hemacytometer.

The data shown in Fig. **5** indicate that treatment with DD-*M. tuberculosis* and DD-*M. smegmatis* reduces the accumulation of lung eosinophils similar to the reduction following immunization with DD-*M. vaccae*, and that DD-*M. tuberculosis* and DD-*M. smegmatis* may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract. Administration of DD-*M. tuberculosis* and DD-*M. smegmatis* may therefore reduce the severity of asthma and diseases that involve similar immune abnormalities, such as allergic rhinitis.

EXAMPLE 7

EFFECT OF DD-*M. VACCAE* ON CYTOKINE PRODUCTION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

This example describes studies on the ability of DD-*M. vaccae* to stimulate production of IL-10, TNF-α and IFN-γ in human peripheral blood mononuclear cells (PBMC).

Human blood was separated into PBMC and non-adherent cells, and the cytokine production of each fraction determined after stimulation with DD-*M. vaccae* as follows. Blood was diluted with an equal volume of saline and 15-20 ml was layered onto 10 ml Ficoll (Gibco BRL Life Technologies, Gaithersburg, MD). The lymphocyte layer was removed after centrifugation at 1,800 rpm for 20 min, washed three times in RPMI medium (Gibco BRL) and counted using Trypan blue. Cells were resuspended in RPMI containing 5% heat-inactivated autologous serum at a concentration of $2x10^6$ per ml. The cell sample was divided to prepare non-adherent cells.

Non-adherent cells were prepared by incubating 20 ml of the lymphocytes in RPMI supplemented with serum (as above) for one hour in a humidified atmosphere containing 5% CO_2. The non-adherent cells were transferred to a fresh flask and the incubation repeated once more. The non-adherent cells were removed, counted and resuspended at a concentration of 2×10^6 per ml in supplemented RPMI medium. Serial dilutions of DD-*M. vaccae* were prepared starting at 200 µg/ml and added to 100 µl medium (supplemented RPMI) in a 96-well plate. PBMC and non-adherent cells were added to the wells (100 µl) and the plates incubated at 37°C for 48 hours in a humidified atmosphere containing 5% CO_2. A

150 µl aliquot was removed from each well to determine the amount of cytokine produced by the different cells after stimulation with DD-*M. vaccae*.

DD-*M. vaccae* stimulated PBMC to secrete TNF-α and IL-10 (Figs. **6** and 7A, respectively), but stimulated the non-adherent cells to produce IFN-γ (Fig. 7B). These data suggest that IFN-γ production in DD-*M. vaccae*-stimulated PBMC is repressed by the simultaneous secretion of IL-10.

EXAMPLE 8

EFFECT OF INTRADERMAL INJECTION OF HEAT-KILLED *MYCOBACTERIUM VACCAE* ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of heat-killed *Mycobacterium vaccae* on psoriasis.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5g/l; tryptone, 5g/l; glucose, 1 g/l) at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI, USA) with glucose at 37 °C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120 °C and stored frozen at –20 °C. Prior to use the *M. vaccae* suspension was thawed, diluted to a concentration of 5 mg/ml in phosphate buffered saline, autoclaved for 15 min at 120 °C and 0.2 ml aliquoted under sterile conditions into vials for use in patients.

Twenty four volunteer psoriatic patients, male and female, 15-61 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-35. The PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body. A PASI score of above 12 reflects widespread disease lesions on the body. The study commenced with a washout period of four weeks where the patients did not have systemic anti-psoriasis treatment or effective topical therapy.

The 24 patients were then injected intradermally with 0.1 ml *M. vaccae* (equivalent to 500 μg). This was followed three weeks later with a second intradermal injection with the same dose of *M. vaccae* (500 μg). Psoriasis was evaluated from four weeks before the first injection of heat-killed *M. vaccae* to twelve weeks after the first injection as follows:

A. The PASI scores were determined at -4, 0, 3, 6 and 12 weeks;

B. Patient questionnaires were completed at 0, 3, 6 and 12 weeks; and

C. Psoriatic lesions: each patient was photographed at 0, 3, 6, 9 and 12 weeks.

The data shown in Table 6 describe the age, sex and clinical background of each patient.

TABLE 6

Patient Data in the Study of the Effect of *M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-001	D.C.	49/F	30 years	28.8
PS-002	E.S.	41/F	4 months	19.2
PS-003	M.G.	24/F	8 months	18.5
PS-004	D.B.	54/M	2 years	12.2
PS-005	C.E.	58/F	3 months	30.5
PS-006	M.G.	18/F	3 years	15.0
PS-007	L.M.	27/M	3 years	19.0
PS-008	C.C	21/F	1 month	12.2
PS-009	E.G	42/F	5 months	12.6
PS-010	J.G	28/M	7 years	19.4
PS-011	J.U	39/M	1 year	15.5
PS-012	C.S	47/M	3 years	30.9
PS-013	H.B	44/M	10 years	30.4
PS-014	N.J	41/M	17 years	26.7
PS-015	J.T	61/F	15 years	19.5
PS-016	L.P	44/M	5 years	30.2
PS-017	E.N	45/M	5 years	19.5

PS-018	E.L	28/F	19 years	16.0
PS-019	B.A	38/M	17 years	12.3
PS-020	P.P	58/F	1 year	13.6
PS-021	L.I	27/F	8 months	22.0
PS-022	A.C	20/F	7 months	26.5
PS-023	C.A	61/F	10 years	12.6
PS-024	F.T	39/M	15 years	29.5

All patients demonstrated a non-ulcerated, localized erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 7, below, are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first and second injections of heat-killed *M. vaccae*. The data shown in Table 8, below, are the PASI scores of the patients at the time of the first injection of *M. vaccae* (Day 0) and 3, 6, 9, 12 and 24 weeks later.

It can clearly be seen that, by week 9 after the first injection of *M. vaccae*, 16 of 24 patients showed a significant improvement in PASI scores. Seven of 14 patients who completed 24 weeks of follow-up remained stable with no clinical sign of redevelopment of severe disease. These results demonstrate the effectiveness of multiple intradermal injections of inactivated *M. vaccae* in the treatment of psoriasis. PASI scores below 10 reflect widespread healing of lesions. Histopathology of skin biopsies indicated that normal skin structure is being restored. Only one of the first seven patients who completed 28 weeks follow-up had a relapse.

TABLE 7

Skin Reaction Measurements in Millimeter

Code No.	**Time of Measurement**					
	First Injection			**Second Injection**		
	48 hours	**72 hours**	**7 days**	**48 hours**	**72 hours**	**7 days**
PS-001	12x10	12x10	10x8	15x14	15x14	10x10
PS-002	18x14	20x18	18x14	16x12	18x12	15x10
PS-003	10x10	14x10	10x8	15x12	15x10	10x10

Code No.	Time of Measurement					
	First Injection			Second Injection		
	48 hours	72 hours	7 days	48 hours	72 hours	7 days
PS-004	14x12	22x18	20x15	20x20	20x18	14x10
PS-005	10x10	13x10	DNR	DNR	DNR	DNR
PS-006	10x8	10x10	6x4	12x10	15x15	10x6
PS-007	15x15	18x16	12x10	15x13	15x12	12x10
PS-008	18x18	13x12	12x10	18x17	15x10	15x10
PS-009	13x13	18x15	12x8	15x13	12x12	12x7
PS-010	13x11	15x15	8x8	12x12	12x12	5x5
PS-011	17x13	14x12	12x11	12x10	12x10	12x10
PS-012	17x12	15x12	9x9	10x10	10x6	8x6
PS-013	18x11	15x11	15x10	15x10	15x13	14x6
PS-014	15x12	15x11	15x10	13x12	14x10	8x5
PS-015	15x12	16x12	15x10	7x6	14x12	6x4
PS-016	6x5	6x6	6x5	8x8	9x8	9x6
PS-017	20x15	15x14	14x10	15x15	17x16	DNR
PS-018	14x10	10x8	10x8	12x12	10x10	10x10
PS-019	10x10	14x12	10x8	DNR	15x14	15x14
PS-020	15x12	15x15	12x15	15x15	14x12	13x12
PS-021	15x12	15x12	7x4	11x10	11x10	11x8
PS-022	12x10	10x8	10x8	15x12	13x10	10x8
PS-023	13x12	14x12	10x10	17x17	15x15	DNR
PS-024	10x10	10x10	10x8	10x8	8x7	8x7

DNR = Did not report.

TABLE 8

Clinical Status of Patients after Injection of *M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 9	Week 12	Week 24
PS-001	28.8	14.5	10.7	2.2	0.7	0
PS-002	19.2	14.6	13.6	10.9	6.2	0.6
PS-003	18.5	17.2	10.5	2.7	1.6	0
Code No.	**Day 0**	**Week 3**	**Week 6**	**Week 9**	**Week 12**	**Week 24**
PS-004	12.2	13.4	12.7	7.0	1.8	0.2
PS-005*	30.5	DNR	18.7	DNR	DNR	0
PS-006	15.0	16.8	16.4	2.7	2.1	3.0
PS-007	19.0	15.7	11.6	5.6	2.2	0
PS-008	12.2	11.6	11.2	11.2	5.6	0
PS-009	12.6	13.4	13.9	14.4	15.3	13.0
PS-010	18.2	16.0	19.4	17.2	16.9	19.3
PS-011	17.2	16.9	16.7	16.5	16.5	15.5
PS-012	30.9	36.4	29.7	39.8**		
PS-013	19.5	19.2	18.9	17.8	14.7	17.8
PS-014	26.7	14.7	7.4	5.8	9.9	24.4***
PS-015	30.4	29.5	28.6	28.5	28.2	24.3
PS-016	30.2	16.8	5.7	3.2	0.8	
PS-017	12.3	12.6	12.6	12.6	8.2	
PS-018	16.0	13.6	13.4	13.4	13.2	
PS-019	19.5	11.6	7.0	DNR	DNR	
PS-020	13.6	13.5	12.4	12.7	12.4	
PS-021	22.0	20.2	11.8	11.4	15.5	
PS-022	26.5	25.8	20.7	11.1	8.3	
PS-023	12.6	9.2	6.6	5.0	4.8	
PS-024	29.5	27.5	20.9	19.0	29.8	

- * Patient PS-005 received only one dose of autoclaved *M. vaccae.*
- ** Patient PS-012 removed from trial, drug (penicillin) induced dermatitis
- *** Patient PS-014 was revaccinated
- DNR = Did not report
- Blank cells indicate pending follow-up

EXAMPLE 9

EFFECT OF INTRADERMAL INJECTION OF DELIPIDATED AND DEGLYCOLIPIDATED *MYCOBACTERIUM VACCAE* (DD-*M.VACCAE*) ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of DD-*M. vaccae* on psoriasis and the lack of T cell proliferation induced in these patients after treatment with DDMV.

Seventeen volunteer psoriatic patients, male and female, 18-48 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-30. As discussed above, the PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body with a PASI score of above 12 reflecting widespread disease lesions on the body. The study commenced with a washout period of four weeks where the patients did not have systemic anti-psoriasis treatment or effective topical therapy. The 17 patients were then injected intradermally with 0.1 ml DD-*M. vaccae* (equivalent to 100 μg). This was followed three weeks later with a second intradermal injection with the same dose of DD-*M. vaccae* (100 μg).

Psoriasis was evaluated from four weeks before the first injection of *M. vaccae* to 48 weeks after the first injection as follows:

A. the PASI scores were determined at -4, 0, 3, 6, 12, 24, 36 and 48 weeks;

B. patient questionnaires were completed at 0, 3, 6, 9 and 12 weeks, and thereafter every 4 weeks; and

C. psoriatic lesions: each patient was photographed at 0 and 3 weeks, and thereafter at various intervals.

The data shown in Table 9 describe the age, sex and clinical background of each patient.

TABLE 9

Patient Data in the Study of the Effect of DD-*M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-025	A.S	25/F	2 years	12.2
PS-026	M.B	45/F	3 months	14.4
PS-027	A.G	34/M	14 years	24.8
PS-028	E.M	31/M	4 years	18.2
PS-029	A.L	44/M	5 months	18.6
PS-030	V.B	42/M	5years	21.3
PS-031	R.A	18/M	3 months	13.0
PS-032		42/M	23 years	30.0
PS-033		37/F	27 years	15.0
PS-034		42/M	15 years	30.4
PS-035		35/M	6 years	13.2
PS-036		43/M	6 years	19.5
PS-037		35/F	4 years	12.8
PS-038		44/F	7 months	12.6
PS-039		20/F	1 year	16.1
PS-040		28/F	8 months	25.2
PS-041		48/F	10 years	20.0

All patients demonstrated a non-ulcerated, localized erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 10 are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first injection of DD-*M. vaccae,* and 48 hours and 72 hours after the second injection.

TABLE 10

Skin Reaction Measurements in Millimeters

Code No.	Time of Measurement				
	First Injection			Second Injection	
	48 hours	72 hours	7 days	48 hours	72 hours
PS-025	8x8	8x8	3x2	10x10	10x10
PS-026	12x12	12x12	8x8	DNR	14x14
PS-027	9x8	10x10	10x8	9x5	9x8
PS-028	10x10	10x10	10x8	10x10	10x10
PS-029	8x6	8x6	5x5	8x8	8x8
PS-030	14x12	14x14	10x10	12x10	12x10
PS-031	10x10	12x12	10x6	14x12	12x10

DNR = Did not report

The data shown in Table 11 are the PASI scores of the 17 patients at the time of the first injection of DD-*M. vaccae* (Day 0), then 3, 6, 12, 24, 36 and 48 weeks later, when available.

TABLE 11

Clinical Status of Patients after Injection of DD-*M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 12	Week 24	Week 36	Week 48	Repeat treatment
PS-025	12 2	4 1	1.8	1.4	1.7	0.2	15.8	Wk 48
PS-026	14 4	11 8	6.0	6.9	1.4	0.4		
PS-027	24 8	23 3	18.3	9.1	10.6	7.5	1.9	
PS-028	18 2	24 1	28.6*					
PS-029	18.6	9.9	7.4	3.6	0.8	0	0	
PS-030	21.3	15.7	13.9	16.5	18.6	5.8	1.7	
PS-031	13.0	5.1	2.1	1.6	0.3	0	0	
PS-032	30.0	28.0	20	12.4	20.4	19.0	21.5	Wk 44

Code No.	Day 0	Week 3	Week 6	Week 12	Week 24	Week 36	Week 48	Repeat treatment
PS-033	19.0	12.6	5.9	4.0	12.6	21.1 (wk 40)	7.1 (wk 52)	Wk 20
PS-034	30.4	31.2	31.6	32.4	25.5	33.0		Wk 20
PS-035	13.2	11.6	10.6	1.6	1.4 (wk 20)	1.0		
PS-036	19.5	18.0	18.0	16.8	18.0	10.2		Wk 20, 32
PS-037	12.8	13.1	1.2	0	0	0		
PS-038	12.6	12.6	12.7	10.0				Wk 12
PS-039	16.1	17.9	18.3	17.0				Wk 12
PS-040	25.2	3.9	0.5					
PS-041	20.0	12.7	0.8					

- * Patient PS-28 removed from trial, exfoliative dermatitis/psoriasis
- Blank cells indicate pending follow-up
- Wk – weeks after first injection

These results show the significant improvement in PASI scores in 16 patients after injection with DD-*M. vaccae*. One patient dropped out of the study at 12 weeks with the diagnosis of exfoliative dermatitis/psoriasis. Patients who relapsed received a second or third injection of DD-*M. vaccae* at the time indicated in Table 11.

At 6 weeks follow-up (n=17), the PASI score improved by >50% in 9 of 17 (53%) patients. At 12 weeks follow up (n=14), the PASI score improved by >50% in 9 of 14 (64.3%) patients. Seven of these patients showed significant clinical improvement with reduction in PASI score to less than 8. At 24 weeks follow up (n=12), the PASI score improved by >50% in 7 of 12 (58%) patients and at 48 weeks follow up (n=7), the PASI score improved by >50% in 5 of 7 (71%) patients. Again, four of these patients showed significant clinical improvement with reduction in PASI score to less than 2. Local injections of DD-M. *vaccae* were observed to result in clearance of skin lesions distant from the site of injection.

Lack of DDMV-specific T-cell proliferative response in peripheral blood cells from patients treated with DDMV

In a lymphocyte proliferation assay, the proliferative effect of DDMV on PBMC from the psoriasis patients after treatment with DDMV was determined. A few of these patients were known to be PPD (purified protein derivative from *M. bovis*) skin test positive and their T cells were shown to proliferate in response to PPD. Donor PBMCs were cultured in medium comprising RPMI 1640 supplemented with 10% (v/v) autologous serum, penicillin (60 mg/ml), streptomycin (100 mg/ml), and glutamine (2 mM) with DDMV (12.5 and 6.25 μg), or heat killed *M.vaccae* (6.25, 12.5, 25 or 50μg/ml) or PPD (10 or 1 μg).

The plates were cultured for 7 days and then pulsed with 1mCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a scintillation counter. Fractions that stimulated proliferation in both replicates two-fold greater than the proliferation observed in cells cultured in medium alone were considered positive.

The data in Table 12 shows that treatment with DDMV at 0 weeks did not enhance T cell proliferative response to DDMV nor *M.vaccae* 6 to 15 weeks later. Generally, treatment with DDMV also did not enhance T cell responses to PPD. Cells from all donors did proliferate *in vitro* upon stimulation with a positive mitogen control, phytohemagglutinnin (PHA).

TABLE 12

Induction of T-cell proliferation in peripheral blood cells from patients treated with DDMV.

Patient No	Time after injection	PPD		*M. vaccae*				DDMV		PHA
		10 μg	1 μg	50 μg	25 μg	12.5 μg	6.25 μg	12.5 μg	6.25 μg	10
025	D0	2.6*	1.2	1.2	0.95	1.4	1.1	nd	nd	21
	6 wks	2.8	2.9	1.4	2.0	1.7	1.5	nd	nd	19.8
	13 wks	1.4	1.0	1.5	1.3	1.3	2.3	2.6	1.3	28.4
026	D0	3.4	2.1	1.3	1.1	1.5	1.1	nd	nd	11.4
	6 wks	1.7	1.4	0.98	1.2	1.2	1.3	nd	nd	12
	13 wks	2.0	1.1	0.8	1.1	1.5	1.5	1.3	1.0	29
027	D0	1.2	0.99	0.73	1.0	1.1	1.1	nd	nd	12.4
	6 wks	0.8	0.8	0.61	0.59	0.77	0.74	nd	nd	6.9
	13 wks	0.82	1.0	1.0	0.8	1.0	0.9	0.78	1.1	16.9
028	D0	1.9	1.4	1.0	1.1	1.1	1.1	nd	nd	24.4

Patient No	Time after injection	PPD		*M. vaccae*				DDMV		PHA
		10 µg	1 µg	50 µg	25 µg	12.5 µg	6.25 µg	12.5 µg	6.25 µg	10
	6 wks	1.4	1.0	0.95	0.97	0.8	0.8	nd	nd	14.7
	14 wks	2.0	0.9	0.8	1.0	1.2	1.3	0.8	0.9	156
029	D0	1.2	1.1	1.7	1.5	1.7	1.7	nd	nd	20
	5 wks	nd	nd	nd	nd	nd	nd	nd	nd	ND
	12 wks	3.5	1.1	1.2	1.2	1.3	1.1	1.0	1.1	154
030	D0	2.0	1.2	1.4	1.6	1.2	1.2	nd	nd	21
	5 wks	nd	nd	nd	nd	nd	nd	nd	nd	nd
	12 wks	4.0	2.4	1.8	2.1	0.9	1.0	2.1	1.5	380
031	D0	1.7	1.3	0.88	1.0	0.81	0.92	nd	nd	15
	5 wks	nd	nd	nd	nd	nd	nd	nd	nd	nd
	12 wks	9.3	5.3	1.4	1.1	1.3	0.7	1.5	1.6	329
032	D0	4.8	2.3	1.4	1.3	0.94	1.4	1.8	1.3	98
	6 wks	5.7	1.9	1.9	1.5	1.4	1.0	1.4	1.3	32
	15 wks	2.4	3.3	0.6	0.54	0.7	0.9	1.4	0.9	74
033	D0	0.7	1.0	1.4	0.74	1.7	1.5	1.7	1.4	709
	6 wks	1.3	1.5	1.2	1.1	0.8	1.3	1.1	1.1	168
	12 wks	085	1.1	1.3	1.2	0.96	1.4	1.7	2.1	211
034	D0	3.1	1.2	1.4	1.1	1.0	1.3	1.1	1.0	110
	6 wks	4.0	1.3	0.9	0.8	0.7	0.7	1.7	1.4	213
	12 wks	3.0	0.6	1.4	0.9	0.5	0.5	1.0	0.9	72
035	D0	4.0	1.7	2.5	1.3	1.4	1.4	2.8	1.4	232
	6 wks	3.2	1.5	2.8	1.4	1.6	1.4	1.8	2.6	670
	12 wks	1.2	0.5	0.8	1.1	1.2	0.4	0.9	0.6	38
036	D0	2.3	1.5	1.1	0.7	1.0	0.9	2.1	1.1	182
	6 wks	5.7	4.2	1.6	1.5	1.9	2.6	2.4	1.4	243
	12 wks	5.9	2.1	2.7	1.9	1.7	1.5	2.9	1.56	153
037	D0	3.3	3.2	1.8	1.5	1.2	1.8	1.9	1.5	145
	6 wks	6.8	3.3	1.1	0.8	0.5	0.5	1.1	0.8	82
	12 wks	10.3	3.6	2.9	1.6	1.4	1.4	1.5	2.0	55

- Nd - not done
- Values expressed as Stimulation Index (SI) = cpm from tritiated thymidine uptake in presence of DDMV / cpm in absence of DDMV
- D0 - Blood sample taken prior to first treatment
- Wks – weeks

EXAMPLE 10

IMMUNOGENICITY AND IMMUNOMODULATING PROPERTIES OF RECOMBINANT PROTEINS DERIVED FROM *M. VACCAE* AND DD-*M. VACCAE*

A. INDUCTION OF T CELL PROLIFERATION AND IFN-γ PRODUCTION

The polynucleotide sequences for the *M. vaccae* antigens GV-1/70, GV-1/83, GV-3, GV4P, GV-5, GV-5P, GV-7, GV-9, GV-13, GV-14, GV-22B, GV-23, GV-24B, GV-27, GV-27A, GV-27B, GV-29, GV-33, GV-35, GV-38AP, GV-38BP, GV-40P, GV-41B, GV-42, GV-44 and GV-45 are provided in SEQ ID NO: 1-26, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 27-52, respectively. The isolation of these antigens and additional information and characterization of these antigens is described in U.S. Patent 6,160,093, the disclosure of which is hereby incorporatedherein by reference in its entirety.

The immunogenicity of *Mycobacterium vaccae* recombinant proteins (referred to herein as GV recombinant proteins) was tested by injecting female BALB/cByJ mice in each hind foot-pad with 10 μg of recombinant GV proteins emulsified in incomplete Freund's adjuvant (IFA). Control mice received phosphate buffered saline in IFA. The draining popliteal lymph nodes were excised 10 days later and the cells obtained therefrom were stimulated with the immunizing GV protein and assayed for proliferation by measuring the uptake of tritiated thymidine. The amount of interferon gamma (IFNγ) produced and secreted by these cells into the culture supernatants was assayed by standard enzyme-linked immunoassay.

As shown in Table 13, all GV proteins were found to induce a T cell proliferative response. The lymph node T cells from immunized mice proliferated in response to the specific GV protein used in the immunization. Lymph node cells from non-immunized mice did not proliferate in response to GV proteins. The data in Table 14 showing IFNγ production, indicate that most of the GV proteins stimulated IFNγ production by lymph node cells from mice immunized with the corresponding GV protein. When lymph node cells from non-immunized mice were cultured with individual GV proteins, IFNγ production was not detectable. The GV proteins are thus able to stimulate T cell proliferation and/or IFNγ

production when administered by subcutaneous injection.

TABLE 13

Immunogenic Properties of GV proteins: Proliferation

GV protein	Proliferation (cpm)		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	2	0.08
GV-1/70	31,550 ± 803	19,058 ± 2,449	5,596 ± 686
GV-1/83	18,549 ± 2,716	23,932 ± 1,964	11,787 ± 1,128
GV-3	34,751 ± 1,382	6,379 ± 319	4,590 ± 1,042
GV-4P	26,460 ± 1,877	10,370 ± 667	6,685 ± 673
GV-5	42,418 ± 2,444	23,902 ± 2,312	13,973 ± 772
GV-5P	35,691 ± 159	14,457 ± 1,185	8,340 ± 725
GV-7	38,686 ± 974	22,074 ± 3,698	15,906 ± 1,687
GV-9	30,599 ± 2580	15,260 ± 2,764	4,531 ± 1,240
GV-13	15,296 ± 2,006	7,163 ± 833	3,701 ± 243
GV-14	27,754 ± 1,872	13,001 ± 3,273	9,897 ± 2,833
GV-22B	3,199 ± 771	3,255 ± 386	1,841 ± 318
GV-23	35,598 ± 1,330	15,423 ± 2,858	7,393 ± 2,188
GV-24B	43,678 ± 2,190	30,307 ± 1,533	15,375 ± 2,594
GV-27	18,165 ± 3,300	16,329 ± 1,794	6,107 ± 1,773
GV-27A	23,723 ± 850	6,860 ± 746	4,295 ± 780
GV-27B	31,602 ± 1,939	29,468 ± 3,867	30,306 ± 1,912
GV-29	20,034 ± 3,328	8,107 ± 488	2,982 ± 897
GV-33	41,529 ± 1,919	27,529 ± 1,238	8,764 ± 256
GV-35	29,163 ± 2,693	9,968 ± 314	1,626 ± 406
GV-38AP	28,971 ± 4,499	17,396 ± 878	8,060 ± 810
GV-38BP	19,746 ± 245	11,732 ± 3,207	6,264 ± 875
GV-40P	25,185 ± 2,877	19,292 ± 2,294	10,883 ± 893
GV-41B	24,646 ± 2,714	12,627 ± 3,622	5,772 ± 1,041
GV-42	25,486 ± 3,029	20,591 ± 2,021	13,789 ± 775
GV-44	2,684 ± 1,995	3,577 ± 1,725	1,499 ± 959

GV protein	Proliferation (cpm)		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	2	0.08
GV-45	9,554 ± 482	3,683 ± 1,127	1,497 ± 199

TABLE 14

Immunogenic properties of GV proteins: IFNγ production

GV protein	**IFNγ (ng/ml)**		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	**10**	**2**
GV-1/70	24.39 ± 6.66	6.19 ± 1.42	1.90 ± 0.53
GV-1/83	11.34 ± 5.46	5.36 ± 1.34	2.73 ± 1.55
GV-3	3.46 ± 0.30	1.57 ± 0.04	not detectable
GV-4P	6.48 ± 0.37	3.00 ± 0.52	1.38 ± 0.50
GV-5	4.08 ± 1.41	6.10 ± 2.72	2.35 ± 0.40
GV-5P	34.98 ± 15.26	9.95 ± 3.42	5.68 ± 0.79
GV-7	33.52 ± 3.08	25.47 ± 4.14	9.60 ± 1.74
GV-9	92.27 ± 45.50	88.54 ± 16.48	30.46 ± 1.77
GV-13	11.60 ± 2.89	2.04 ± 0.58	1.46 ± 0.62
GV-14	8.28 ± 1.56	3.19 ± 0.56	0.94 ± 0.24
GV-22B	not detectable	not detectable	not detectable
GV-23	59.67 ± 14.88	30.70 ± 4.48	9.17 ± 1.51
GV-24B	6.76 ± 0.58	3.20 ± 0.50	1.97 ± 0.03
GV-27	72.22 ± 11.14	30.86 ± 10.55	21.38 ± 3.12
GV-27A	4.25 ± 2.32	1.51 ± 0.73	not detectable
GV-27B	87.98 ± 15.78	44.43 ± 8.70	21.49 ± 5.60
GV-29	7.56 ± 2.58	1.22 ± 0.56	not detectable
GV-33	7.71 ± 0.26	8.44 ± 2.35	1.52 ± 0.24
GV-38AP	23.49 ± 5.89	8.87 ± 1.62	4.17 ± 1.72
GV-38BP	5.30 ± 0.95	3.10 ± 1.19	1.91 ± 1.01
GV-40P	15.65 ± 7.89	10.58 ± 1.31	3.57 ± 1.53

GV protein	IFNγ (ng/ml)		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	10	2
GV-41B	16.73 ± 1.61	5.08 ± 1.08	2.13 ± 1.10
GV-42	95.97 ± 23.86	52.88 ± 5.79	30.06 ± 8.94
GV-44	not detectable	not detectable	not detectable

B. ACTIVATION OF LYMPHOCYTE SUBPOPULATIONS

The ability of recombinant *M. vaccae* proteins, heat-killed *M. vaccae* and DD-*M. vaccae* to activate lymphocyte subpopulations was determined by examining upregulation of expression of CD69 (a surface protein expressed on activated cells).

PBMC from normal donors (5 x 10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae* or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 24 hours. CD69 expression was determined by staining cultured cells with monoclonal antibody against CD56, αβT cells or γδT cells in combination with monoclonal antibodies against CD69, followed by flow cytometry analysis

Table 15 shows the percentage of αβT cells, γδT cells and NK cells expressing CD69 following stimulation with heat-killed *M. vaccae*, DD-*M. vaccae* or recombinant *M. vaccae* proteins. These results demonstrate that heat-killed *M. vaccae*, DD-*M. vaccae* and GV-23 stimulate the expression of CD69 in the lymphocyte subpopulations tested compared with control (non-stimulated cells), with particularly high levels of CD69 expression being seen in NK cells. GV-45 was found to upregulate CD69 expression in αβT cells.

TABLE 15

Stimulation of CD69 Expression

	αβT cells	γδT cells	NK cells
Control	3.8	6.2	4.8
Heat-killed *M. vaccae*	8.3	10.2	40.3
DD-*M. vaccae*	10.1	17.5	49.9

	αβT cells	γδT cells	NK cells
GV-22B	5.6	3.9	8.6
GV-23	5.8	10.0	46.8
GV-27	5.5	4.4	13.3
GV-27A	5.5	4.4	13.3
GV-27B	4.4	2.8	7.1
GV-45	11.7	4.9	6.3

The ability of the recombinant protein GV-23 (20 μg/ml) to induce CD69 expression in lymphocyte subpopulations was compared with that of the known Th1-inducing adjuvants MPL/TDM/CWS (Monophosphoryl Lipid A/Trehalose 6'6' dimycolate- Sigma, St. Louis, MO at a final dilution of 1:20/cell wall skeleton: mycolic acid-arabino-galactan-mucopeptide) and CpG ODN (oligodeoxynucleotide-Promega, Madison, WI; 20 μg/ml), and the known Th2-inducing adjuvants aluminium hydroxide (Superfos Biosector, Kvistgard, Denmark; at a final dilution of 1:400) and cholera toxin (20 μg/ml), using the procedure described above. MPL/TDM/CWS and aluminium hydroxide were employed at the maximum concentration that does not cause cell cytotoxicity. Figs. **8**A-C show the stimulation of CD69 expression on αβT cells, γδT cells and NK cells, respectively. GV-23, MPL/TDM/CWS and CpG ODN induced CD69 expression on NK cells, whereas aluminium hydroxide and cholera toxin did not.

C. STIMULATION OF CYTOKINE PRODUCTION

The ability of recombinant *M. vaccae* proteins to stimulate cytokine production in PBMC was examined as follows. PBMC from normal donors (5 x 10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 24 hours. Culture supernatants were harvested and tested for the production of IL-1β, TNF-α, IL-12 and IFN-γ using standard ELISA kits (Genzyme, Cambridge, MA), following the manufacturer's instructions. Figs. **9**A-D show the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively. Heat-killed *M. vaccae* and DD-*M. vaccae* were found to stimulate the production of all four

cytokines examined, while recombinant GV-23 and GV-45 were found to stimulate the production of IL-1β, TNF-α and IL-12. Figs. 10A-C show the stimulation of IL-1β, TNF-α and IL-12 production, respectively, in human PBMC (determined as described above) by varying concentrations of GV-23 and GV-45.

Figs. 11A-D show the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively, in PBMC by GV-23 as compared to that by the adjuvants MPL/TDM/CWS (at a final dilution of 1:20), CpG ODN (20 μg/ml), aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 μg/ml). GV-23, MPL/TDM/CWS and CpG ODN induced significant levels of the four cytokines examined, with higher levels of IL-1β production being seen with GV-23 than with any of the known adjuvants. Aluminium hydroxide and cholera toxin induced only negligible amounts of the four cytokines.

D. ACTIVATION OF ANTIGEN PRESENTING CELLS

The ability of heat-killed *M. vaccae*, DD-*M. vaccae* and recombinant *M. vaccae* proteins to enhance the expression of the co-stimulatory molecules CD40, CD80 and CD86 on B cells, monocytes and dendritic cells was examined as follows.

Peripheral blood mononuclear cells depleted of T cells and comprising mainly B cells, monocytes and dendritic cells were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B, GV-23, GV-27, GV27A, GV-27B or GV-45 for 48 hours. Stimulated cells were harvested and analyzed for up-regulation of CD40, CD80 and CD86 using 3 color flow cytometric analysis. Tables 16, 17 and 18 show the fold increase in mean fluorescence intensity from control (non-stimulated cells) for dendritic cells, monocytes, and B cells, respectively.

TABLE 16

Stimulation of CD40, CD80 and CD86 Expression on Dendritic Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	6.1	3.8	1.6
DD-*M. vaccae*	6.6	4.2	1.6
GV-22B	4.6	1.9	1.6
GV-23	6.0	4.5	1.8
GV-27	5.2	1.9	1.6
GV-27A	2.3	0.9	1.0
GV-27B	2.6	1.1	1.1
GV-45	5.8	3.0	3.1

TABLE 17

Stimulation of CD40, CD80 and CD86 Expression on Monocytes

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	2.3	1.8	0.7
DD-*M. vaccae*	1.9	1.5	0.7
GV-22B	0.7	0.9	1.1
GV-23	2.3	1.5	0.7
GV-27	1.5	1.4	1.2
GV-27A	1.4	1.4	1.4
GV-27B	1.6	1.2	1.2
GV-45	1.6	1.2	1.0

TABLE 18

Stimulation of CD40, CD80 and CD86 Expression on B Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	1.6	1.0	1.7
DD-*M. vaccae*	1.5	0.9	1.7
GV-22B	1.1	0.9	1.2
GV-23	1.2	1.1	1.4
GV-27	1.1	0.9	1.1
GV-27A	1.0	1.1	0.9
GV-27B	1.0	0.9	0.9
GV-45	1.2	1.1	1.3

As shown above, increased levels of CD40, CD80 and CD86 expression were seen in dendritic cells, monocytes and B cells with all the compositions tested. Expression levels were most increased in dendritic cells, with the highest levels of expression being obtained with heat-killed *M. vaccae*, DD-*M. vaccae*, GV-23 and GV-45. Figs. 12A-C show the stimulation of expression of CD40, CD80 and CD86, respectively, in dendritic cells by varying concentrations of GV-23 and GV-45.

The ability of GV-23 to stimulate CD40, CD80 and CD86 expression in dendritic cells was compared to that of the Th1-inducing adjuvants MPL/TDM/CWS (at a final dilution of 1:20) and CpG ODN (20 μg/ml), and the known Th2-inducing adjuvants aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 μg/ml). GV23, MPL/TDM/CWS and CpG ODN caused significant up-regulation of CD40, CD80 and CD86, whereas cholera toxin and aluminium hydroxide induced modest or negligible dendritic cell activation, respectively.

E. DENDRITIC CELL MATURATION AND FUNCTION

The effect of the recombinant *M. vaccae* protein GV-23 on the maturation and function of dendritic cells was examined as follows.

Purified dendritic cells (5 x 10^4 – 10^5 cells/ml) were stimulated with GV-23 (20 μg/ml) or LPS (10 μg/ml) as a positive control. Cells were cultured for 20 hour and then analyzed for CD83 (a maturation marker) and CD80 expression by flow cytometry. Non-stimulated cells were used as a negative control. The results are shown below in Table 19.

TABLE 19

Stimulation of CD83 Expression in Dendritic Cells

Treatments	% CD83-positive dendritic cells	% CD80-positive dendritic cells
Control	15 ± 8	9 ± 6.6
GV-23	35 ± 13.2	24.7 ± 14.2
LPS	36.3 ± 14.8	27.7 ± 13

Data = mean± SD (n=3)

The ability of GV-23 to enhance dendritic cell function as antigen presenting cells was determined by mixed lymphocyte reaction (MLR) assay. Purified dendritic cells were cultured in medium alone or with GV-23 (20 μg/ml) for 18-20 hours and then stimulated with allogeneic T cells (2 x 10^5 cells/well). After 3 days of incubation, (^{3}H)-thymidine was added. Cells were harvested 1 day later and the uptake of radioactivity was measured. Fig. 13 shows the increase in uptake of (^{3}H)-thymidine with increase in the ratio of dendritic cells to T cells. Significantly higher levels of radioactivity uptake were seen in GV-23 stimulated dendritic cells compared to non-stimulated cells, showing that GV-23 enhances dendritic cell mixed lymphocyte reaction.

EXAMPLE 11

EFFECT OF INTRAPERITONEAL ADMINISTRATION OF AVAC ON THE EXPRESSION OF GENES INVOLVED IN NOTCH SIGNALING IN MICE

The capacity of AVAC to modulate expression of genes involved in Notch signaling was assessed in 6-week-old female BALB/cByJ mice as follows. On day 0, mice were immunized intraperitoneally (i.p.) with a mixture containing 10 μg ovalbumin adsorbed to 1 mg aluminium hydroxide adjuvant (Alum, Alu-Gel-S, Serva), or with OVA-Alum mixture to which was added 1 mg AVAC, using 10 mice per group. On day 7, all mice were immunized i.p. with OVA-Alum only. Ten days later, all mice were sacrificed. The spleen was removed from each animal, pooled with other spleens from the same treatment group, and cell suspensions prepared. $CD4^+$ cells were isolated from each pooled spleen cell suspension using a Mouse T Cell CD4 Subset Kit (R&D Systems, Minneapolis MN). The cells, >75% CD4+ as determined by flow cytometry using FITC-conjugated rat anti-mouse CD4 monoclonal antibody (clone GK1.5, Pharmingen), were then stored in TRIZOL™ (Invitrogen) at –80°C. RNA was extracted as per the manufacturer's instructions, and 1 μg of purified RNA was transcribed into cDNA using Superscript (Invitrogen), and subjected to real-time PCR analysis using an ABI Prism 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems, Foster City, CA). Primers and fluorogenic probes were specific for human Notch1, Notch2, Notch3, Delta1, Delta3, Serrate1, Serrate2, HES1, HES5, and Deltex.

As shown in Fig. 14, real-time PCR analysis revealed that treatment of mice with AVAC caused striking increases in expression of Notch receptors, ligands, and downstream targets. Relative expression of Notch receptors ranged from 8-fold (Notch3) up to 22-fold (Notch1). With the exception of Delta1 (<2-fold), relative expression of Notch ligands ranged from almost 15-fold (Delta3, Serrate2) to >100-fold (Serrate1). Relative expression of downstream Notch signaling targets ranged from 2-fold (HES1) to 6-fold (Deltex).

In subsequent experiments, the ability of AVAC to modulate expression of the Notch signaling genes HES5, Lunatic Fringe and Deltex, as well as the cytokines IL-2, IL-4, IL-5, IL-13, IL-12p35, IL-12p40, IL-10, TGFbeta1, IFN-gamma and CD86, as examined essentially as described above. As shown in Fig. 17, real-time PCR analysis revealed that

treatment of mice with AVAC caused suppression of IL-4 (3.5 fold), IL-5 (7 fold) and IL-13 (15 fold) gene expression. These gene products are required for allergic sensitization and are Th2 type cytokines.

EXAMPLE 12

EFFECT OF INTRANASAL ADMINISTRATION OF AVAC and DD-*M. VACCAE* ON EXPRESSION OF GENES INVOLVED IN NOTCH SIGNALING IN MICE

The ability of DD-*M. vaccae* and AVAC to modulate expression of genes involved in Notch signaling was assessed in 6-week-old female BALB/cByJ mice as follows.

Three mice per group were immunized intranasally with 50 µl PBS containing 1 mg AVAC or 1 mg DD-*M. vaccae*. Mice were sacrificed 24 hours later and lung samples from the mice were snap-frozen in liquid nitrogen for RNA extraction. Samples from individual animals were pooled into treatment groups and lung tissues were homogenized. Total RNA was extracted using Trizol reagent, 1 µg. of purified RNA transcribed into cDNA using Superscript First Strand Synthesis System (Invitrogen), and subjected to real-time PCR analysis using an ABI Prism 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems, Foster City, CA). Primers and fluorogenic probes were specific for human Notch1, Notch2, Notch3, Notch4, Delta4, HES5 and Deltex, as well as the cytokines TGFbeta1, IL-2 and IL-10.

As shown in Fig. 16, real-time PCR analysis revealed that treatment of mice with AVAC and DD-*M. vaccae* (referred to as PVAC in Fig. 16) caused TGFβ1 gene expression to be significantly induced in comparison to the control group. Significant IL-10 gene induction was also found in both treatment groups. TGFβ1 and IL-10 are considered to be anti-inflammatory. HES-5 gene expression was suppressed in the AVAC treated group (~4 fold) and was not detectable in the DD-*M. vaccae* treated group. Deltex gene expression was suppressed in the presence of AVAC and DD-*M. vaccae*.

EXAMPLE 13

EFFECT OF *M. VACCAE*, DD-*M. VACCAE*, AVAC AND *M. VACCAE* GLYCOLIPIDS ON EXPRESSION OF CYTOKINES AND GENES INVOLVED IN NOTCH SIGNALING IN HUMAN CELLS

The ability of inactivated *M. vaccae*, DD-*M. vaccae*, AVAC and *M. vaccae* glycolipids to modulate expression of genes involved in Notch signaling, cytokines and Toll-like receptors (TLR) was assessed as follows using the human myelomonocytic cell line THP-1 (American Type Culture Collection, Manassas, VA).

THP-1 cells were maintained in RPMI (Gibco BRL Life Technologies) supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). For assay, THP-1 cells were resuspended at 1 x 10^6/ml in cRPMI-5 in a volume of 4 ml in 6-well plates. After saving an aliquot of THP-1 cells for reference purposes (t=0 hr baseline control), inactivated *M. vaccae, DD-M. vaccae*, AVAC or *M. vaccae* glycolipids was added to the cell suspension to achieve a final concentration of 100 µg/ml. The cells were subsequently cultured in a humidified 37°C incubator supplied with a gas mixture of 5% CO_2 in air. Cells were collected at various time points (3, 6, 12 and 24 hours), centrifuged, resuspended in TRIZOL™ (Gibco BRL Life Technologies), and frozen at –80°C. RNA was extracted as per the manufacturer's instructions, and 1 µg of purified RNA was transcribed into cDNA using Superscript First Strand Synthesis System (Invitrogen, Carlsbad, CA), and the cDNA subjected to real-time PCR analysis using an ABI Prism 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems, Foster City, CA). Primers and fluorogenic probes were specific for the Notch signaling genes human Notch1, Notch2, Notch3, Notch4, Deltex, Jagged-1, Jagged-2, Delta-like 1, Delta-like 3, HES-1, HERP1, HERP2, Lunatic Fringe, Manic Fringe, Radical Fringe, Numb, MAML1 and RBP-Jkappa; the Toll-like receptors TLR2, TLR7, TLR8, MyD88 and CD14; and the cytokines IL-12p35, IL-12p40, IL-10, IL-1β, IL-6, IL-8, IL-23p19 and TNFα.

As shown in Fig. 15A-C, IL-10, IL-1β and TNFα gene expression was dramatically upregulated in response to all stimuli. The Notch related genes Lunatic Fringe and HES-1 were dramatically induced (~30 fold) with stimuli showing a dose/response and time

dependent induction of Lunatic Fringe and HES-1 gene expression. Deltex gene expression was also upregulated by these stimuli but was below detection limits in the absence of stimuli. There was a trend towards Notch-1 (3-4 fold) and Notch-3 (2.5-8 fold) upregulation and Notch 4 downregulation (-3 to –7 fold).

Table 20 summarizes the effects of inactivated *M. vaccae*, DD-*M. vaccae*, AVAC, and *M. vaccae* glycolipids on the expression of genes involved in Notch signalingin THP-1 cells.

TABLE 20

	Relative expression*				
Notch signaling gene	*M. vaccae*	DD-*M. vaccae*	AVAC	Glycolipids	LPS
Notch1	1.90	1.60	3.20	1.90	2.30
Notch2	1.40	1.10	1.40	1.20	1.40
Notch3	5.00	-	15.1	1.90	2.30
Notch4	0.06	0.16	0.14	0.24	0.10
Jagged1	1.80	1.30	1.10	2.20	1.70
Jagged2	0.31	0.90	0.90	0.34	0.54
Delta1	7.20	1.20	2.50	0.90	0.80
Delta-like3	0.47	1.20	1.00	1.50	1.20
Delta-like4	134.8	64.6	46.4	25.5	41.6
HES1	57.0	71.0	140.0	22.0	49.0
Deltex	7.00	5.50	11.70	2.70	1.00
HERP1	-	-	-	-	-
HERP2	7.00	2.30	4.50	0.69	1.00
Lunatic fringe	12.0	9.00	18.0	7.50	4.00
Manic fringe	0.38	0.67	0.30	0.59	0.45
Radical fringe	0.65	0.89	0.92	0.80	0.67
Presenilin1	1.39	1.37	0.85	1.54	1.28
Numb	1.89	1.29	1.26	0.92	0.74
MAML1	1.06	1.27	0.90	0.96	0.67
RBP-Jκ	0.78	1.21	0.94	0.62	0.56
HASH1	0.16	0.23	0.31	0.15	1.00

*Normalized relative expression of target gene mRNA in stimulus vs. medium control samples at t=24 hr.

As shown in Table 20, *M. vaccae* upregulated Notch3, Delta1, Delta-like4, HES1, Deltex, HERP2, and Lunatic fringe expression; DD-*M. vaccae* upregulated Delta-like4, HES1, Deltex and Lunatic fringe expression; AVAC upregulated Notch1, Notch3, (Delta1), Delta-like4, HES1, Deltex, HERP2 and Lunatic fringe expression; and *M. vaccae* glycolipids upregulated Delta-like4, HES1, Deltex and Lunatic fringe expression. *M. vaccae* down-regulated Notch4, Jagged2, Manic fringe and HASH1 expression; DD-*M. vaccae* down-regulated Notch4 and HASH1; AVAC down-regulated Notch4, Manic fringe and HASH1 expression and *M. vaccae* glycolipids down-regulated Notch4, Jagged2 and HASH1 expression.

A summary of the effects of inactivated *M. vaccae*, DD-*M. vaccae*, AVAC, and *M. vaccae* glycolipids on the expression of cytokines in THP-1 cells is presented in Table 21.

TABLE 21

	Relative expression*				
Cytokine gene	*M. vaccae*	DD-*M. vaccae*	AVAC	Glycolipids	LPS
IL-1β	4939	1097	2759	4011	246
IL-6	260	125	130	11.6	27.1
IL-8	3769	695	1722	284	267
IL-10	391	17.6	47.5	11.2	8.6
IL-12p35	0.21	0.08	0.10	0.05	0.19
IL-12p40	576	14.8	2684	115	311
IL-23p19	198	93.0	252	18.0	8.0
TNFα	10.3	4.1	5.3	4.7	5.7

*Normalized relative expression of target gene mRNA in stimulus vs. medium control samples at t=24 hr.

As shown in Table 21, *M. vaccae* upregulated IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNFα expression; DD-*M. vaccae* upregulated IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNFα expression; AVAC upregulated IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-23p19 and TNFα expression; and *M. vaccae* glycolipids upregulated IL-1β, IL-6, IL-8, IL-10,

IL-12p40, IL-23p19 and TNFα expression. *M. vaccae* downregulated IL-12p35; DD-*M. vaccae* downregulated IL-12p35; AVAC downregulated IL-12p35; and *M. vaccae* glycolipids downregulated IL-12p35 expression.

In further studies, the production of IL-12p40 protein in THP-1 cells in response to increasing concentrations of heat-killed *M. vaccae*, DD-*M. vaccae*, AVAC and *M. vaccae* glycolipids was examined by ELISA as described above. As shown in Fig. 18, production of IL-12p40 was found to increase with increasing concentrations of *M. vaccae* derivatives.

The differential effect of *M. vaccae* derivatives on IL-12 and IL-23 gene expression in THP-1 cells was examined using real-time PCR as follows.

THP-1 cells were maintained in RPMI (Gibco BRL Life Technologies) supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). THP-1 cells were cultured with 100 μg/mL heat-killed *M. vaccae*, 100 μg/mL DD-*M. vaccae*, 100 μg/mL AVAC, with *M. vaccae* glycolipids, or with no *M. vaccae* derivative for 24 hours in cell culture medium in 6-well tissue culture plates at $1x10^6$ cells/mL in a final volume of 4.0 mL cRPMI-10 (or $4x10^6$ cells per well) in a water-jacketed, humidified incubator at 37°C and supplied with 5% CO_2 in air. At the end of the 24-hour incubation period, the cells were collected and centrifuged at 200xg for 5 minutes, and the supernatants transferred to sterile 10-ml tubes. 1.0 ml Trizol Reagent (Gibco cat. no. 15596-018) were added to each well to lyse the cells. The resulting mixture in each well was then transferred to a sterile 1.8-ml cyrovial and stored at –80°C.

Isolation of RNA for synthesis of cDNA was performed as described in the protocol supplied with the Trizol Reagent. RNA isolated as above was treated with DNase1 (1 U/mL, Invitrogen cat. no. 18008-015). Synthesis of cDNA was then performed as described in the protocol supplied with the First Strand cDNA Synthesis Kit (Invitrogen cat. no. 11904-018).

Forward and reverse primers were designed using Perkin Elmer/Applied Biosystems (ABI) Primer Express software. Real-time PCR was performed using methodology reported by Lin Yin et al (*Immunol Cell Biol* 79:213-221, 2001) and amplification curves plotted using the ABI 7700 Sequence Detection System (Perkin Elmer/Applied Biosystems). Expression

data obtained for THP-1 cells cultured with *M. vaccae* derivatives were normalized to levels observed for THP-1 cells cultured in cRPMI-10 only, and the normalized values plotted as relative expression levels. As shown in Fig. 19, AVAC, DD-*M. vaccae*, heat-killed *M. vaccae* and *M. vaccae* glycolipids were shown to induce expression of IL-12p40 and IL-23p19 mRNA and to suppress expression of IL-12p35 mRNA.

EXAMPLE 14

EFFECT OF *M. VACCAE*, DD-*M. VACCAE*, AVAC AND *M. VACCAE* GLYCOLIPIDS ON TOLL-LIKE RECEPTOR SIGNALING IN HUMAN CELLS

Since the Toll-like receptor TLR2 is known to mediate biological effects of mycobacteria and their products, particularly cell wall components, and since DD-*M. vaccae* and AVAC contain at least one known TLR2 ligand, namely peptidoglycan, the effect of *M. vaccae* derivatives on the expression of TLR genes in THP-1 cells was examined essentially as described above using primers and fluorogenic probes specific for the TLR signaling genes CD14, TLR2, TLR7, TLR8 and MyD88. A summary of the effects of inactivated *M. vaccae*, DD-*M. vaccae*, AVAC, and *M. vaccae* glycolipids on TLR signaling in THP-1 cells is presented in Table 22.

TABLE 22

	Relative expression*				
TLR signaling gene	*M. vaccae*	DD-*M. vaccae*	AVAC	Glycolipids	LPS
CD14	44.5	48.6	68.3	26.7	16.3
TLR2	1.9	2.0	1.0	1.7	1.7
TLR7	2.0	5.5	1.7	11.4	4.2
TLR8	42.6	77.2	133.4	67.6	42.1
MyD88	3.2	2.5	1.6	1.1	3.3

*Normalized relative expression of target gene mRNA in stimulus vs. medium control samples at t=24 hr.

These results demonstrate that *M. vaccae* upregulated CD14 and MyD88 expression; DD-*M. vaccae* upregulated CD14, TLR7 and TLR8 expression; AVAC upregulated CD14, TLR8 expression; and *M. vaccae* glycolipids upregulated CD14, TLR7 and TLR8 expression.

In subsequent experiments, the effect of antibodies to TLR2, TLR4 and CD14 on the production of IL-12p40, IL-10 and TNF-α in THP-1 cells in response to *M. vaccae* derivatives was examined as follows.

THP-1 cells were maintained in RPMI (Gibco BRL Life Technologies) supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). Prior to culture with *M. vaccae* derivatives, 50 µL of THP-1 cells in cRPMI-10 were pre-treated in duplicate microplate wells with 50 µL of serially diluted Functional Grade mAbs to human TLR2 (clone TL2.1, IgG2a isotype, eBioscience cat. no. 16-9922-82), TLR4 (clone HTA125, IgG2a isotype, eBioscience cat. no. 16-9927-82), or CD14 (clone RM052, IgG2a isotype, Coulter cat. no. IM0643), with a cocktail of all three antibodies or with control mAb (clone AcV1, IgG2a isotype, eBioscience cat. no. 16-4724-85), with each mAb used at a final concentration of 1000 µg/mL, 200 µg/mL, 40 µg/mL, 8.0 µg/mL, 1.60 µg/mL, or 0.32 µg/mL, or with no mAb. Pretreatment of cells with mAbs was for 60 minutes in a water-jacketed, humidified incubator at 37°C supplied with 5% CO_2 in air.

Following pretreatment with mAbs, THP-1 cells were cultured with 5 µg/mL heat-killed *M. vaccae* (MV), 5 µg/mL DD-*M. vaccae*, 5 µg/mL AVAC, or with no *M. vaccae* derivative for 24 hours in cell culture medium in 96-well round-bottom microculture plates at $1x10^6$ cells/mL in a final volume of 0.2 mL cRPMI-10 (or $2x10^5$ cells per microwell) in a water-jacketed, humidified incubator at 37°C and supplied with 5% CO_2 in air. At the end of the 24-hour incubation period, the microplates were centrifuged at 200xg for 5 minutes and the supernatants collected and transferred to a sterile 96-well round-bottom plate.

IL-12p40, TNFα, and IL-10 content in the microculture supernatants was determined by sandwich ELISA using commercially available sets according to the manufacturer's recommendations. For IL-12p40, supernatants were diluted 1:2 in cRPMI-10 prior to analysis and the sensitivity of the ELISA was 4 pg IL-12p40 per mL. For TNFα, supernatants were diluted 1:5 in cRPMI-10 prior to analysis and the sensitivity of the ELISA was 8.0 pg TNFα

per mL. For IL-10, supernatants were diluted 1:2 in cRPMI-10 prior to analysis and the sensitivity of the ELISA was 2.0 pg IL-10 per mL.

The production of IL-12p40 by THP-1 cells cultured with neutralizing antibodies and either heat-killed *M. vaccae*, DD-*M. vaccae* or AVAC is shown in Figs. 20A-C, respectively. These figures show that *M. vaccae*-, AVAC- and DD-*M. vaccae*-induced production of IL-12p40 is inhibited by TLR2 and CD14 mAbs in a dose-dependent fashion. The production of TNFα by THP-1 cells cultured with neutralizing antibodies and either heat-killed *M. vaccae*, DD-*M. vaccae* or LPS is shown in Figs. 21A-C, respectively. Fig. 22 shows the production of IL-10 by THP-1 cells cultured with neutralizing antibodies and heat-killed *M. vaccae*. These results provide evidence that *M. vaccae* derivatives elicit production of cytokines through Toll-like receptor signaling.

EXAMPLE 15

EFFECT OF *M. VACCAE*, DD-*M. VACCAE*, AVAC AND *M. VACCAE* GLYCOLIPIDS ON MRP8 SIGNALING IN HUMAN CELLS

The effect of *M. vaccae* derivatives on MRP8 (S100A8) signaling in THP-1 cells was determined essentially as described above using primers and fluorogenic probes for MRP8. The results are shown in Table 23.

TABLE 23

Relative expression of MRP8				
M. vaccae	DD-*M.vaccae*	AVAC	Glycolipids	LPS
44.5	48.6	68.3	26.7	16.3

*Normalized relative expression of MRP8 gene mRNA in stimulus vs. medium control samples at t=24 hr.

These results demonstrate that *M. vaccae*, DD-*M. vaccae*, AVAC, *M. vaccae* glycolipids all upregulate expression of MRP8 (S100A8). MRP-8 is a calcium-binding protein associated with psoriasis and other inflammatory skin disorders. A causal relationship between MRP-8 expression and disease has not yet been established

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EXAMPLE 16

INVOLVEMENT OF MAP KINASE SIGNALING IN PRODUCTION OF CYTOKINES IN HUMAN CELLS IN RESPONSE TO AVAC

The involvement of the MAP kinase signaling pathway in the production of IL-10 by

10 THP-1 cells in response to AVAC was assessed as follows.

THP-1 cells were maintained in RPMI (Gibco BRL Life Technologies) supplemented with antibiotics, L-glutamine, 2-mercaptoethanol, and 5% fetal calf serum (cRPMI-5). Prior to culture with AVAC, 50 µL of THP-1 cells in cRPMI-10 were pre-treated in duplicate microplate wells with 50 µL of serially diluted PD98059 (Calbiochem cat. no. 51300, a

15 selective inhibitor of MAP kinase), SB202190 (Calbiochem cat. no. 559388, an inhibitor of p38 MAP kinase and p38β MAP kinase), SB203580 (Calbiochem cat. no. 559389, a highly specific inhibitor of p38 MAP kinase), with SB202474 (Calbiochem cat. no. 559387, a negative control for MAP kinase inhibition studies), or with no added chemicals. MAP kinase inhibitors and control were used at a final concentration of 100 µg/mL, 20 µg/mL, 4.0

20 µg/mL, 0.8 µg/mL, 0.16 µg/mL, or 0.032 µM. Pretreatment of cells with MAP kinase inhibitors and control was for 120 minutes in a water-jacketed, humidified incubator at 37°C supplied with 5% CO_2 in air.

Following pretreatment, the cells were washed once in cPRMI-10 to remove inhibitor or control chemicals. The THP-1 cells were then cultured with 25 µg/mL AVAC, or with no

25 *M. vaccae* derivative for 24 hours in cell culture medium in 96-well round-bottom microculture plates at $1x10^6$ cells/mL in a final volume of 0.2 mL cRPMI-10 (or $2x10^5$ cells per microwell) in a water-jacketed, humidified incubator at 37°C and supplied with 5% CO_2 in air. At the end of the 24-hour incubation period, the microplates were centrifuged at 200xg for 5 minutes and the supernatants collected and transferred to a sterile 96-well round-bottom

30 plate. IL-10 content in the microculture supernatants was determined by sandwich ELISA

using a commercially available set (eBioscience cat. no. 88-7106-77,) according to the manufacturer's recommendations. Supernatants were diluted 1:2 in cRPMI-10 prior to analysis. The sensitivity of the ELISA was approximately 2.0 pg IL-10 per mL.

The results of this experiment, expressed in Optical Density (O.D.) values are provided in Fig. 23, and show that production of IL-10 by THP-1 cells cultured with AVAC was substantially suppressed in a dose-dependent manner by the p38 MAP kinase inhibitors SB202190 and SB203580, and to a lesser extent by the MAP kinase inhibitor PD98059. These data indicate that production of IL-10 by THP-1 cells in response to AVAC involves the MAP kinase signaling pathway.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

We claim:

1. A method for modulating the expression of Notch ligands on antigen presenting cells, comprising contacting the antigen presenting cells with a composition comprising at least one component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

2. The method of claim 1, wherein the antigen presenting cellsare dendritic cells.

3. A method for modifying an immune response to an antigen in a subject, comprising administering to the subject a composition comprising at least one component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

4. A method for stimulating infectious tolerance to an antigen in a subject, comprising administering to the subject a composition comprising at least one component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

5. A method for treating a disorder characterized by the presence of an abnormal immune response in a subject, the method comprising administering to the subject a composition comprising at least one component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

6. A method for modulating Notch signaling in a population of cells, comprising contacting the cells with a composition comprising at least one component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

7. A method for modulating Notch signaling in a population of cells, comprising contacting the cells with a composition comprising an isolated polypeptide, wherein the polypeptide comprises a sequence selected from the group consisting of:

(a) SEQ ID NO: 27-52;

(b) sequences encoded by a sequence of SEQ ID NO: 1-26;

(c) sequence having at least 75% identity to a sequence of SEQ ID NO: 27-52; and

(d) sequences having at least 90% identity to a sequence of SEQ ID NO: 27-52.

8. A method for modulating Notch signaling in a population of cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of:

(a) delipidated and deglycolipidated *M. smegmatis* cells; and

(b) delipidated and deglycolipidated *M. tuberculosis* cells.

9. A method for modulating expression of a Notch signaling gene in a population of cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

10. The method of claim 9, wherein the Notch signaling molecule is selected from the group consisting of: Notch1, Notch2, Notch3, Notch4, Deltex, Jagged-1, Jagged-2, Delta-like 1, Delta-like 3, HES-1, HERP1, HERP2, Lunatic Fringe, Manic Fringe, Radical Fringe, Numb, MAML1 and RBP-Jkappa.

11. A method for modulating expression of a Toll-like receptor gene in a population of cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

12. A method for modulating Notch signaling in a population of cells, comprising contacting the cells with a composition comprising peptidoglycan.

13. A method for modulating Toll-like receptor signaling in a population of cells, comprising contacting the cells with a composition comprising peptidoglycan.

14. A method for modulating Toll-like receptor signaling in a population of cells, comprising contacting the cells with a composition comprising a component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis;

(d) delipidated and deglycolipidated *M. vaccae* cells that have been treated by acid hydrolysis;

(e) delipidated and deglycolipidated *M. vaccae* cells that have been treated with periodic acid;

(f) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and by acid hydrolysis;

(g) delipidated and deglycolipidated *M. vaccae* cells that have been treated by alkaline hydrolysis and treated with periodic acid;

(h) delipidated and deglycolipidated *M. vaccae* cells that have been treated with Proteinase K; and

(i) delipidated and deglycolipidated *M. vaccae* cells that have been treated by hydrofluoric acid hydrolysis.

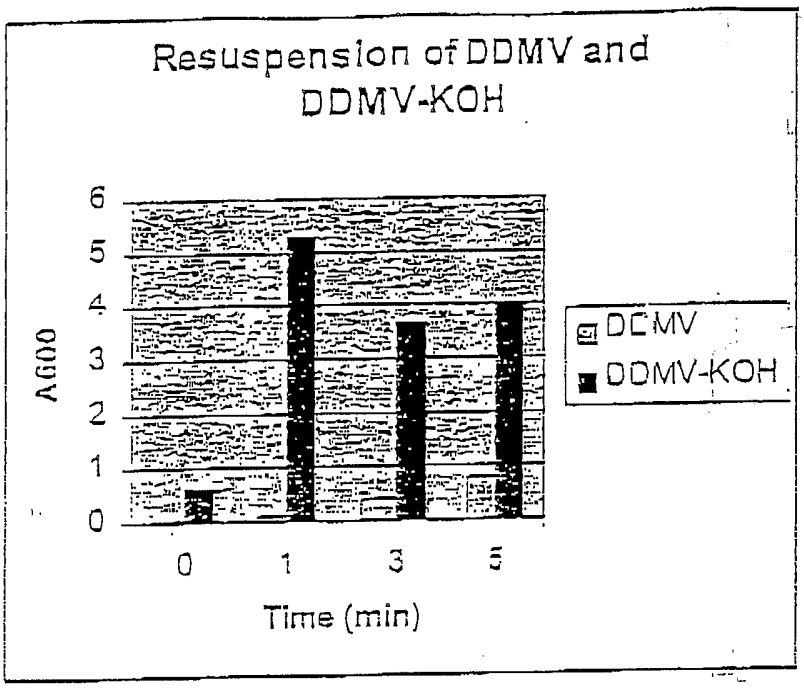

Resuspension of DDMV and DDMV-KOH
A600
6
5
4
3
2
1
0
0
1
3
5
Time (min)
DDMV
DDMV-KOH

FIGURE 1

FIGURE 2

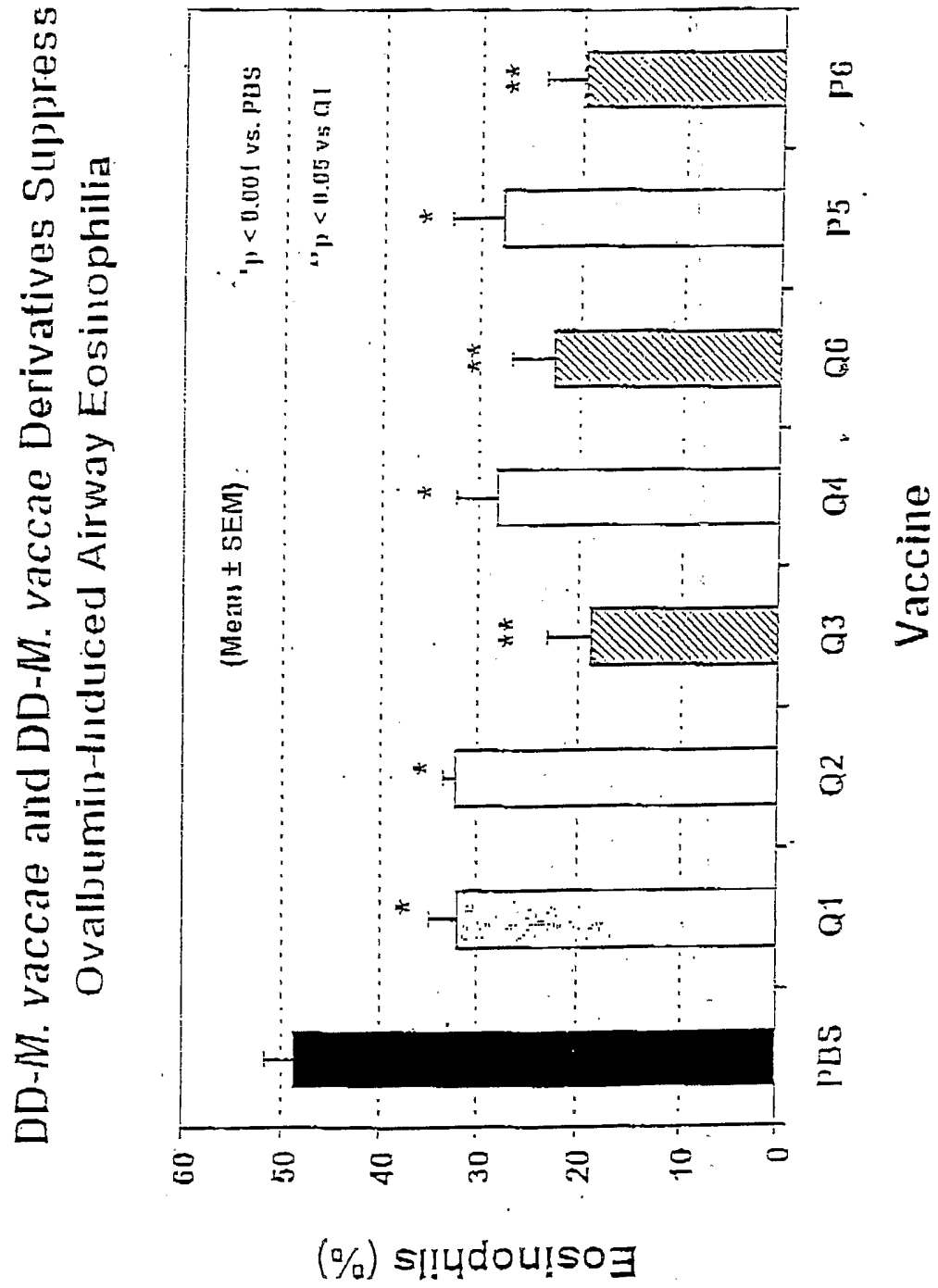

DD-M. vaccae and DD-M. vaccae Derivatives Suppress Ovalbumin-Induced Airway Eosinophilia
(Mean ± SEM)
* p < 0.001 vs. PBS
** p < 0.05 vs Q1
Eosinophils (%)
60
50
40
30
20
10
0
PBS
Q1
Q2
Q3
Q4
Q6
P5
P6
Vaccine

FIGURE 3

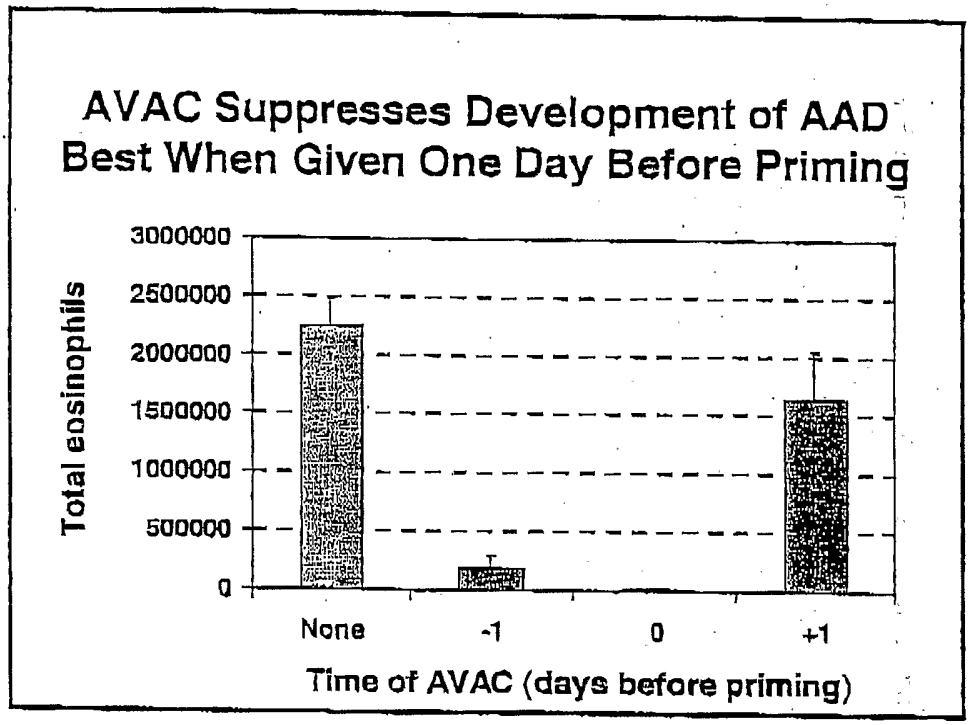

AVAC Suppresses Development of AAD
Best When Given One Day Before Priming
Total eosinophils
3000000
2500000
2000000
1500000
1000000
500000
0
None
-1
0
+1
Time of AVAC (days before priming)

FIGURE 4

IL-10 stimulation of THP-1 cells
by DD-*M. vaccae* and DD-*M. vaccae* derivatives

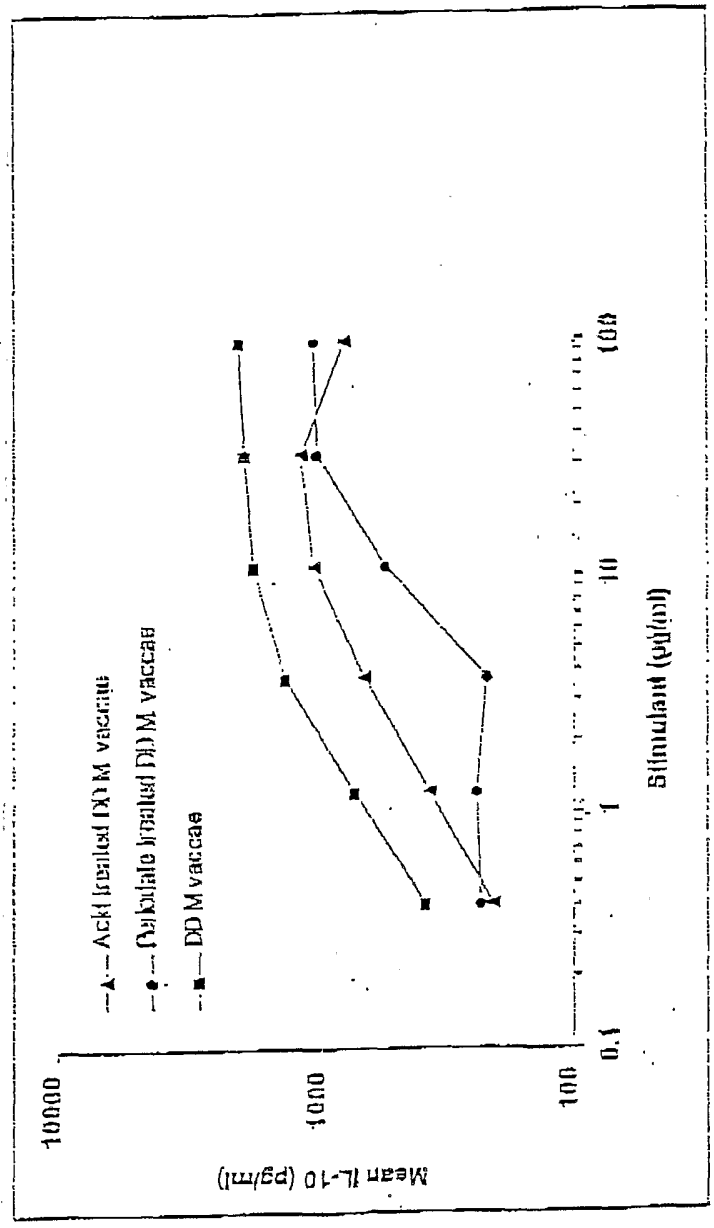


FIGURE 5

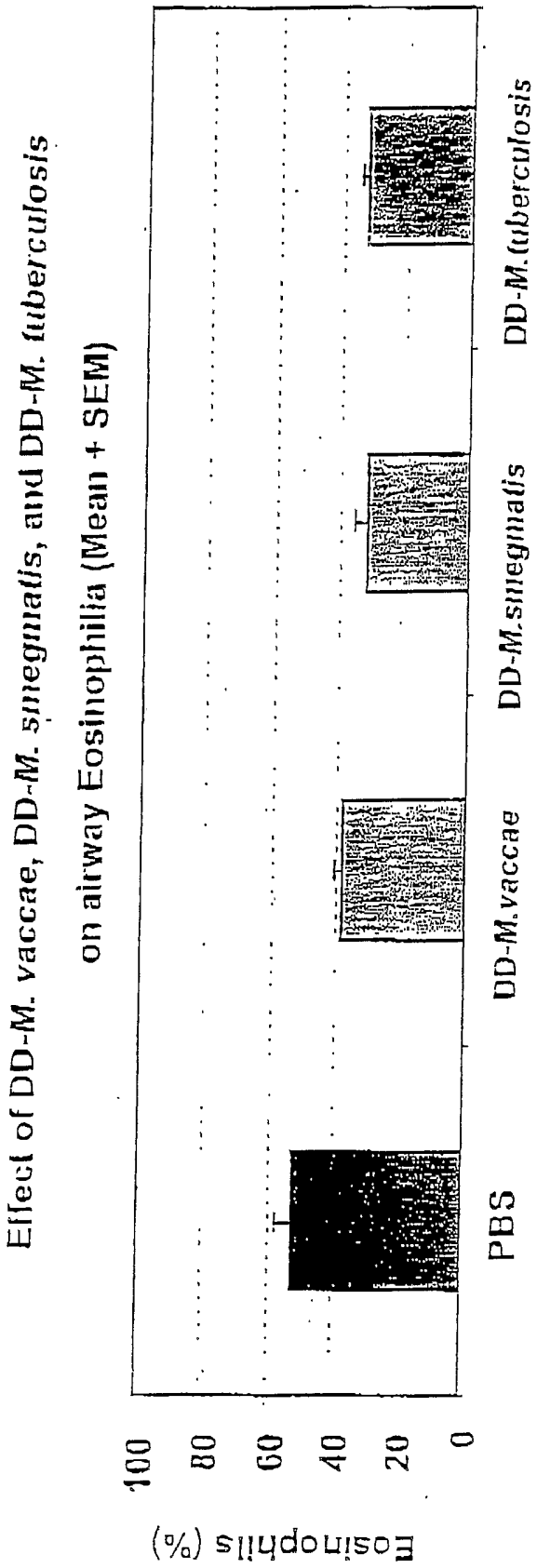
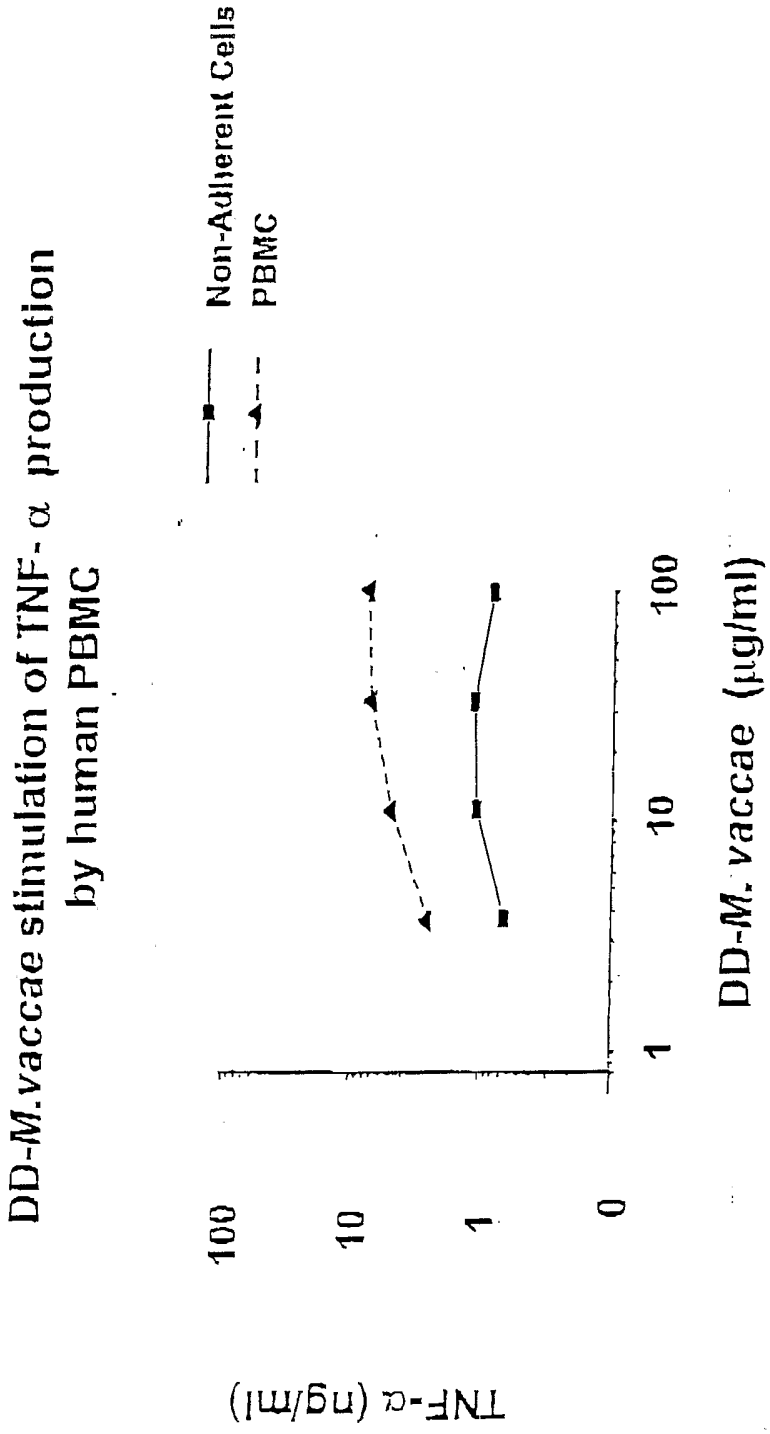

Effect of DD-M. vaccae, DD-M. smegmatis, and DD-M. tuberculosis
on airway Eosinophilia (Mean + SEM)
Eosinophils (%)
100
80
60
40
20
0
PBS
DD-M.vaccae
DD-M.smegmatis
DD-M.tuberculosis
Vaccine

FIGURE 6


DD-M.vaccae stimulation of TNF- α production by human PBMC
Non-Adherent Cells
PBMC
TNF-α (ng/ml)
100
10
1
0
1
10
100
DD-M. vaccae (μg/ml)

DD-*M. vaccae* stimulation of IL-10 and IFN-γ production by human PBMC

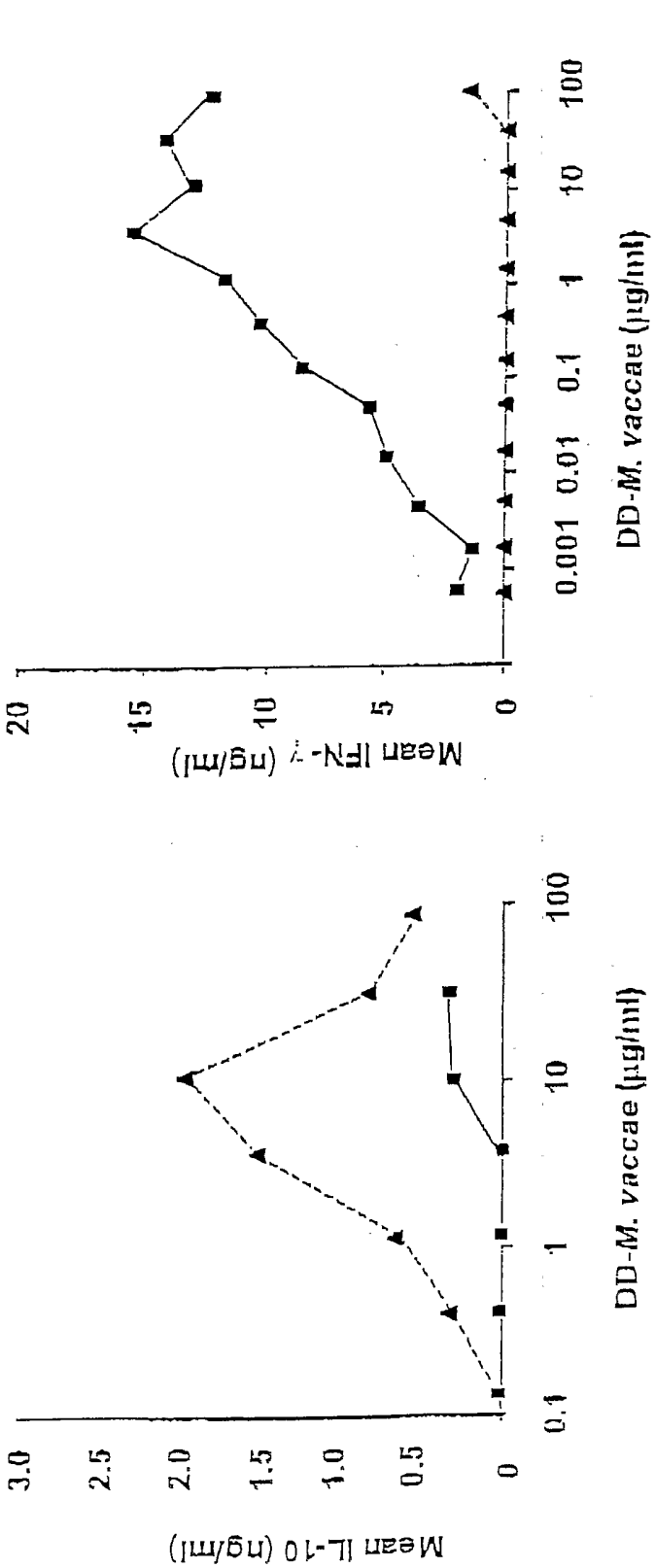


FIGURE 7A

FIGURE 7B

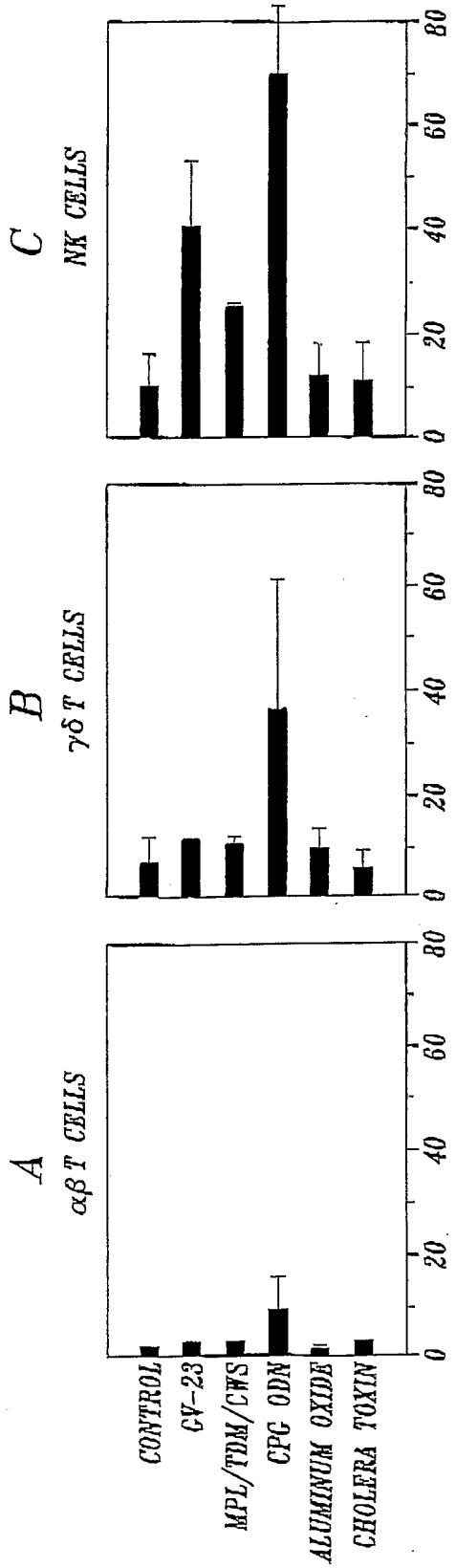

A
αβ T CELLS
B
γδ T CELLS
C
NK CELLS
0
20
40
60
80
CONTROL
CV-23
MPL/TDM/CWS
CPG ODN
ALUMINUM OXIDE
CHOLERA TOXIN
PERCENT OF CELLS EXPRESSING CD69
* BAR = MEAN +/- S.E.
n=2

FIGURE 8

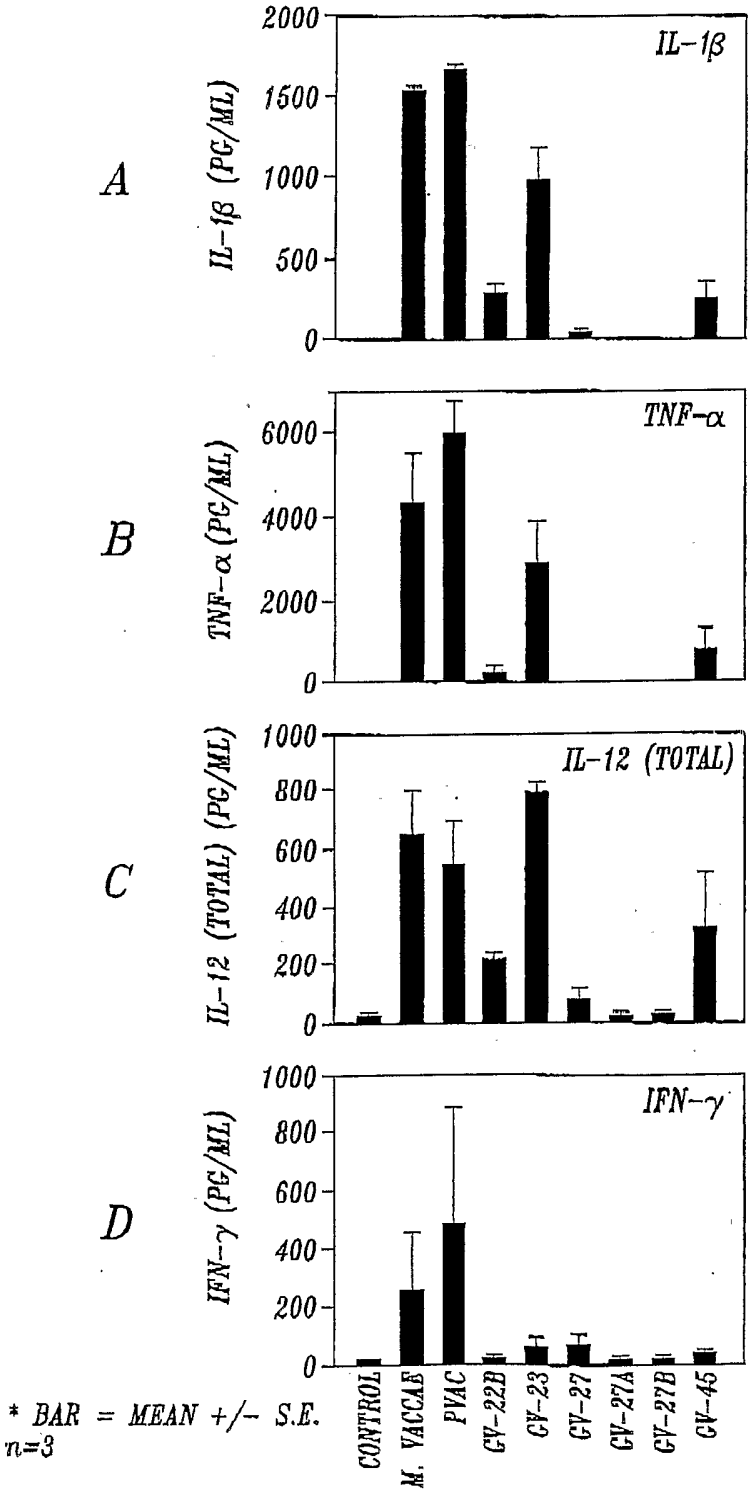

A
IL-1β (PG/ML)
2000
1500
1000
500
0
IL-1β
B
TNF-α (PG/ML)
6000
4000
2000
0
TNF-α
C
IL-12 (TOTAL) (PG/ML)
1000
800
600
400
200
0
IL-12 (TOTAL)
D
IFN-γ (PG/ML)
1000
800
600
400
200
0
IFN-γ
CONTROL
M. VACCAE
PVAC
GV-22B
GV-23
GV-27
GV-27A
GV-27B
GV-45
* BAR = MEAN +/- S.E.
n=3

FIGURE 9

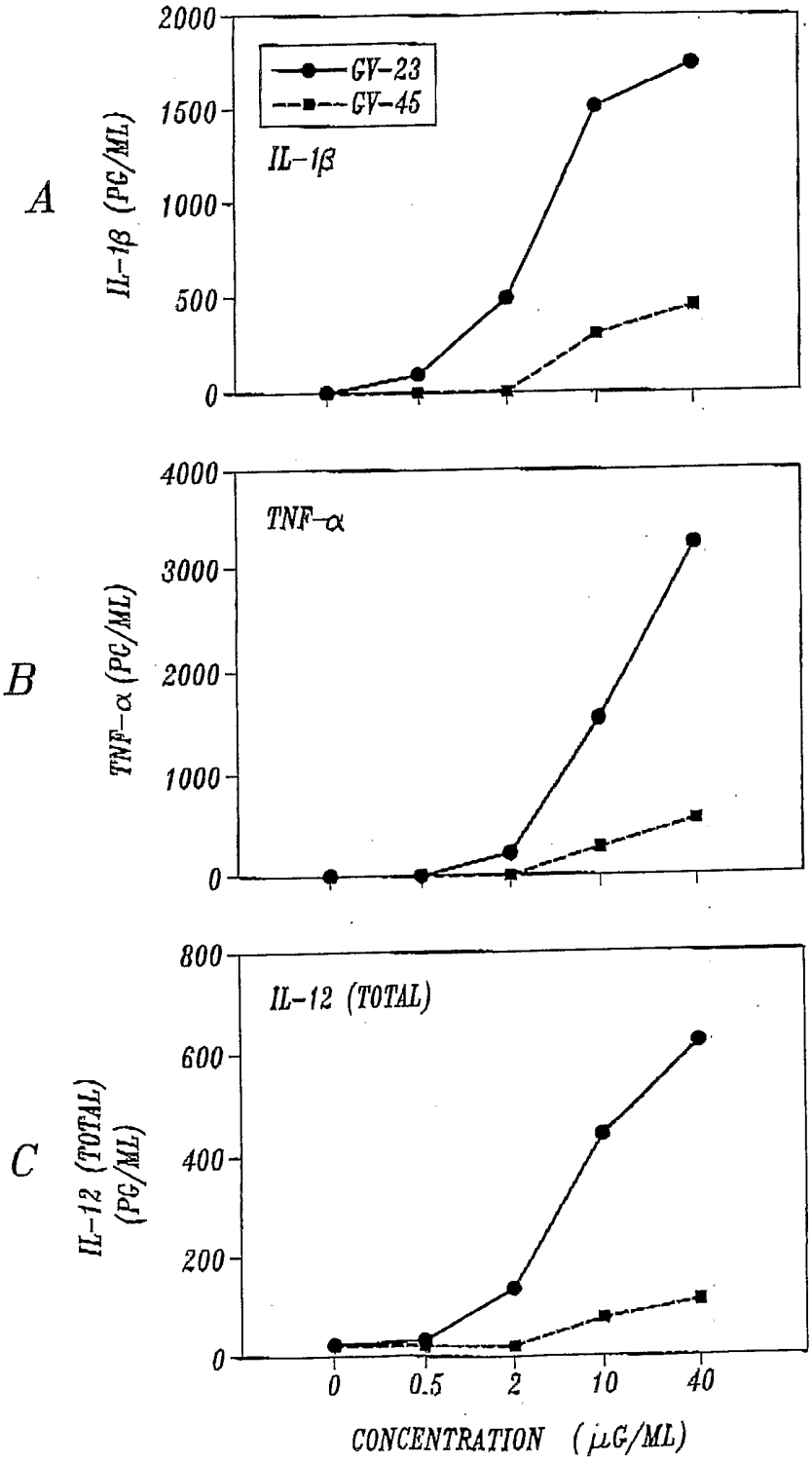

A
B
C
GV-23
GV-45
IL-1β
IL-1β (PG/ML)
2000
1500
1000
500
0
TNF-α
TNF-α (PG/ML)
4000
3000
2000
1000
0
IL-12 (TOTAL)
IL-12 (TOTAL) (PG/ML)
800
600
400
200
0
0
0.5
2
10
40
CONCENTRATION (μG/ML)

FIGURE 10

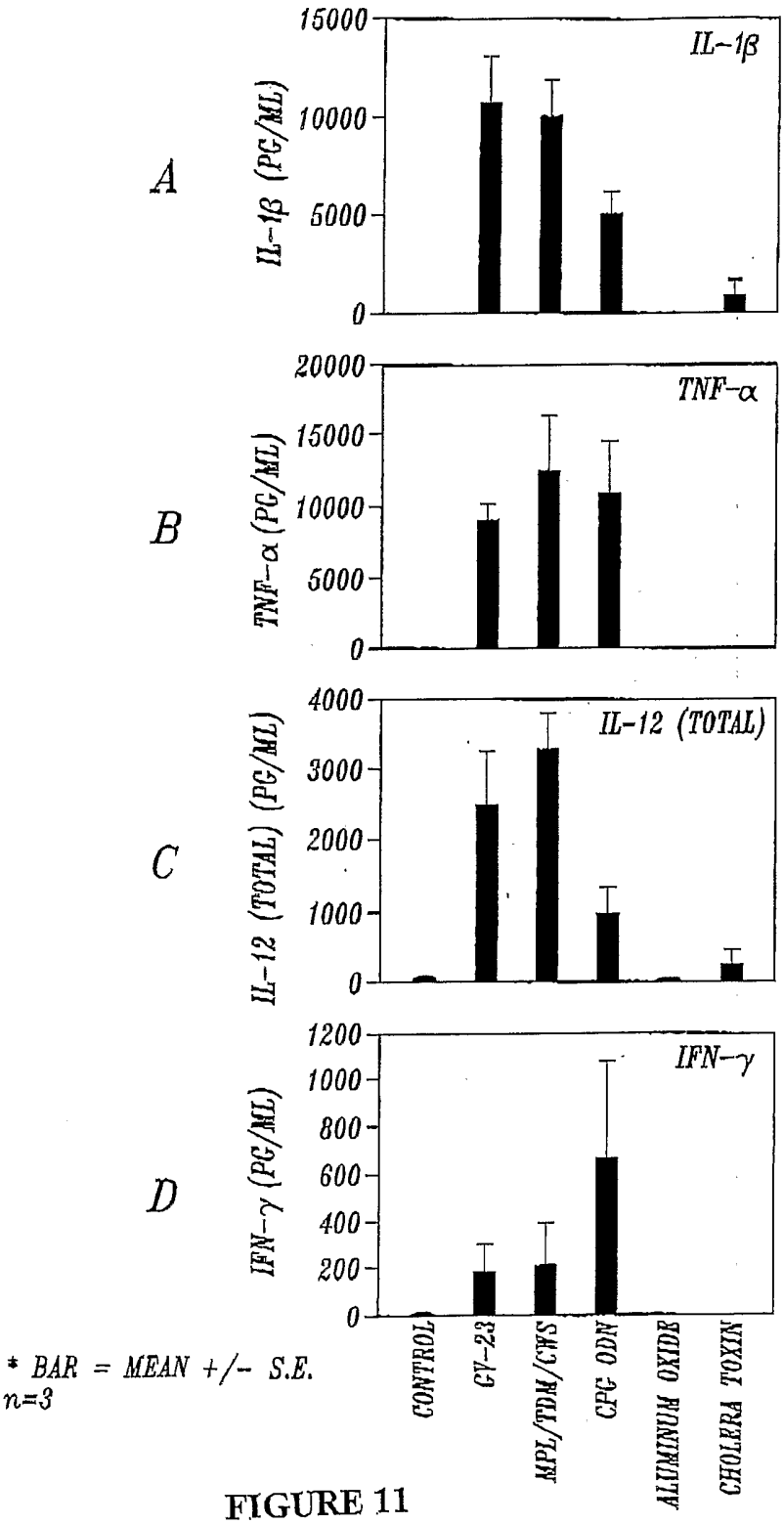

A
IL-1β (PG/ML)
15000
10000
5000
0
IL-1β
B
TNF-α (PG/ML)
20000
15000
10000
5000
0
TNF-α
C
IL-12 (TOTAL) (PG/ML)
4000
3000
2000
1000
0
IL-12 (TOTAL)
D
IFN-γ (PG/ML)
1200
1000
800
600
400
200
0
IFN-γ
CONTROL
CY-23
MPL/TDM/CWS
CPG ODN
ALUMINUM OXIDE
CHOLERA TOXIN
* BAR = MEAN +/- S.E.
n=3

FIGURE 11

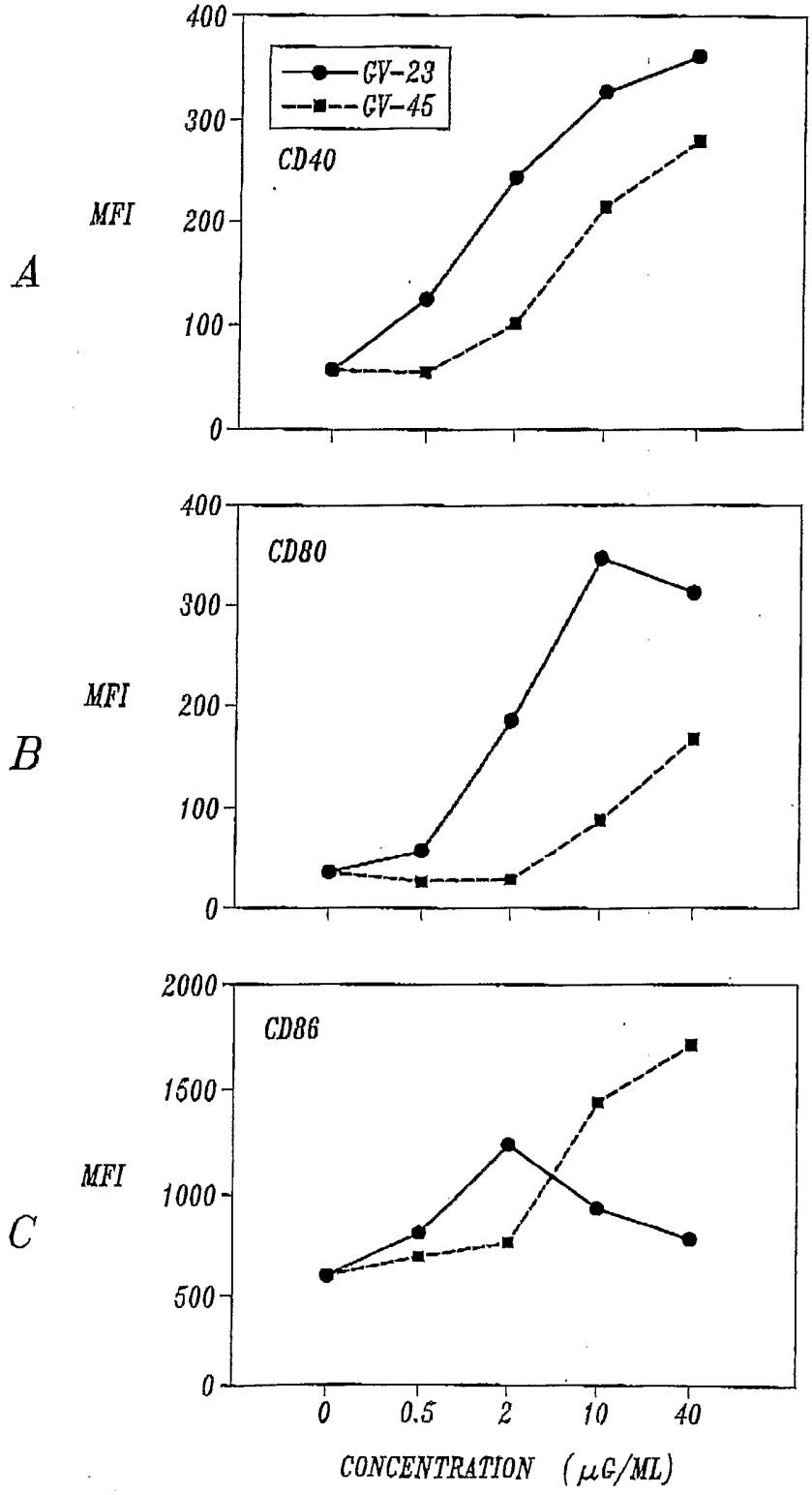

A
MFI
400
300
200
100
0
GV-23
GV-45
CD40
B
MFI
400
300
200
100
0
CD80
C
MFI
2000
1500
1000
500
0
CD86
0
0.5
2
10
40
CONCENTRATION (μG/ML)

FIGURE 12

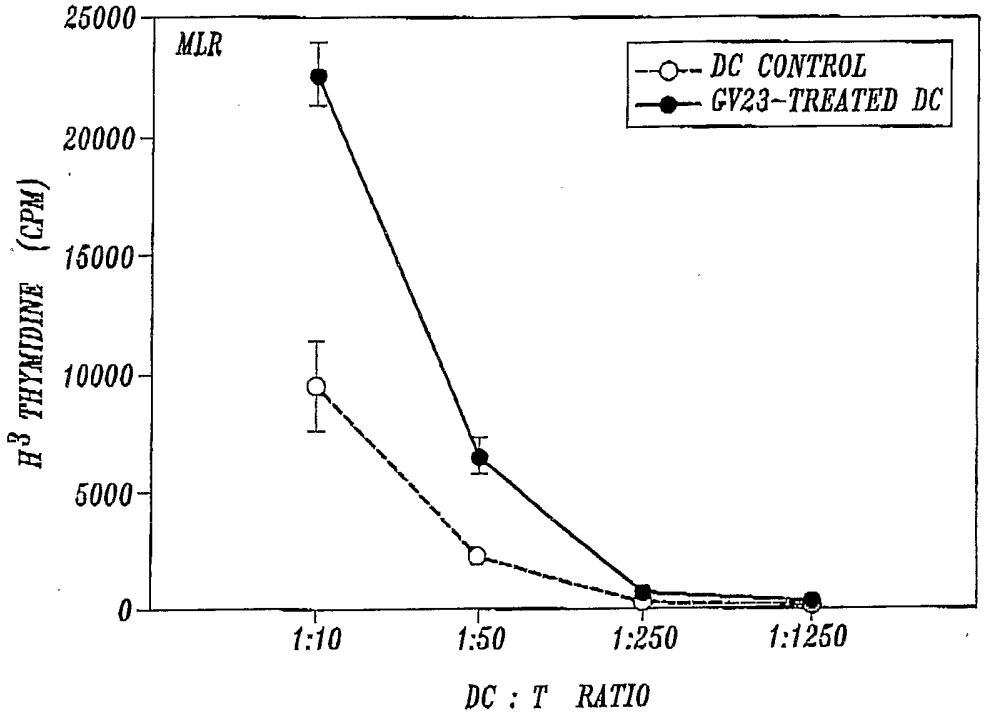

MLR
DC CONTROL
GV23-TREATED DC
25000
20000
15000
10000
5000
0
H3 THYMIDINE (CPM)
1:10
1:50
1:250
1:1250
DC : T RATIO

FIGURE 13

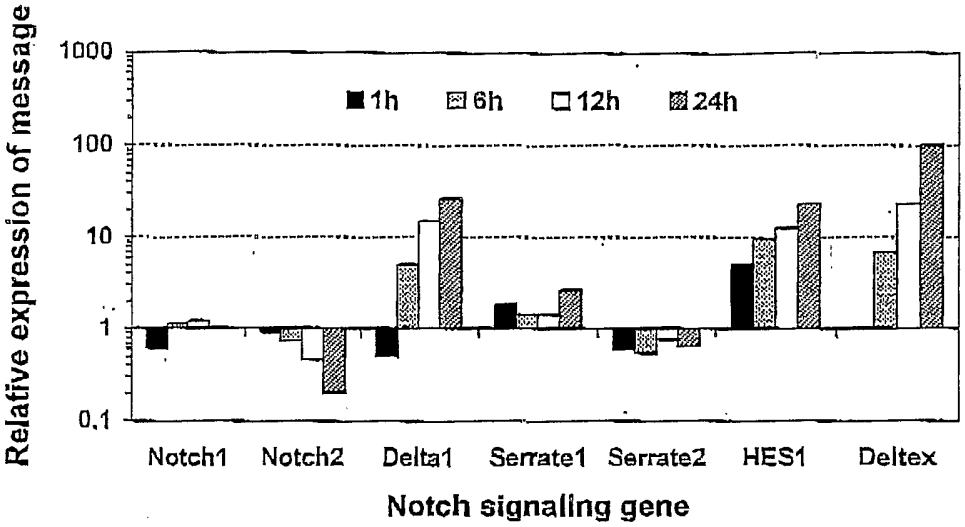

Relative expression of message
1000
100
10
1
0.1
1h
6h
12h
24h
Notch1
Notch2
Delta1
Serrate1
Serrate2
HES1
Deltex
Notch signaling gene

FIGURE 14

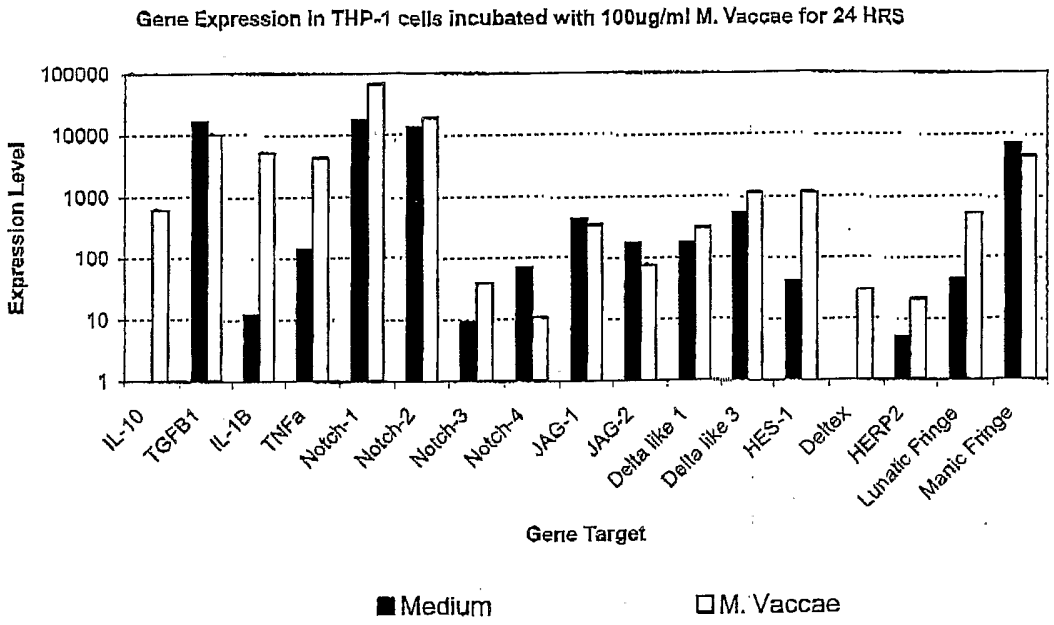

Gene Expression in THP-1 cells incubated with 100ug/ml M. Vaccae for 24 HRS
Expression Level
100000
10000
1000
100
10
1
IL-10
TGFB1
IL-1B
TNFa
Notch-1
Notch-2
Notch-3
Notch-4
JAG-1
JAG-2
Delta like 1
Delta like 3
HES-1
Deltex
HERP2
Lunatic Fringe
Manic Fringe
Gene Target
Medium
M. Vaccae

Figure 15A

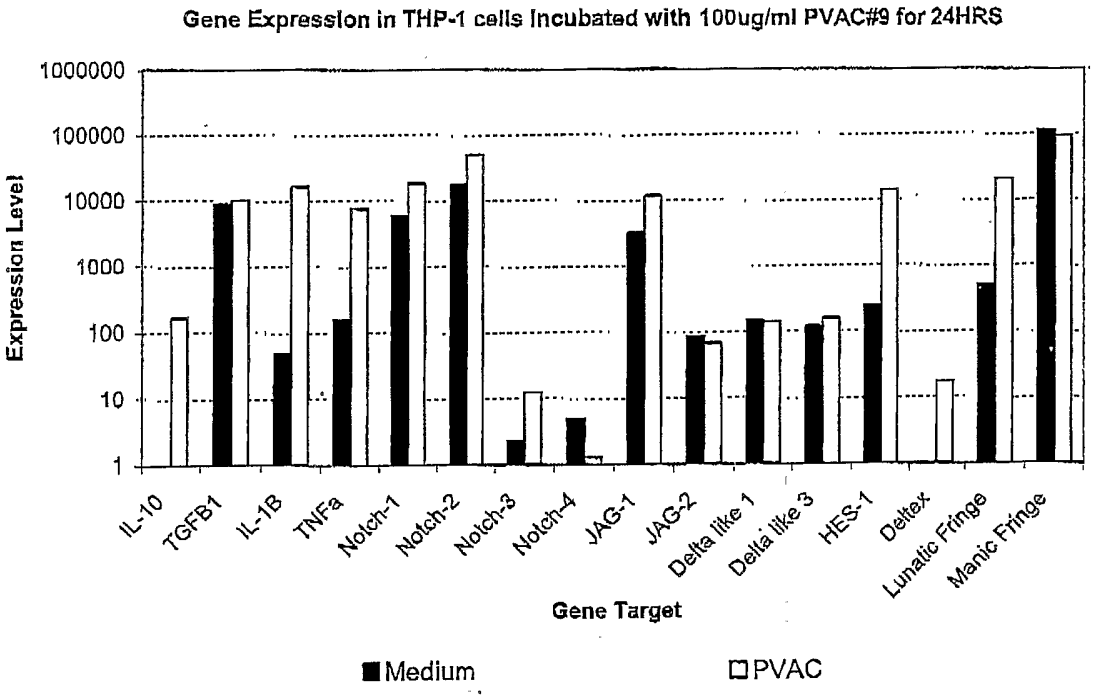

Gene Expression in THP-1 cells Incubated with 100ug/ml PVAC#9 for 24HRS
Expression Level
1000000
100000
10000
1000
100
10
1
IL-10
TGFB1
IL-1B
TNFa
Notch-1
Notch-2
Notch-3
Notch-4
JAG-1
JAG-2
Delta like 1
Delta like 3
HES-1
Deltex
Lunatic Fringe
Manic Fringe
Gene Target
Medium
PVAC

Figure 15B

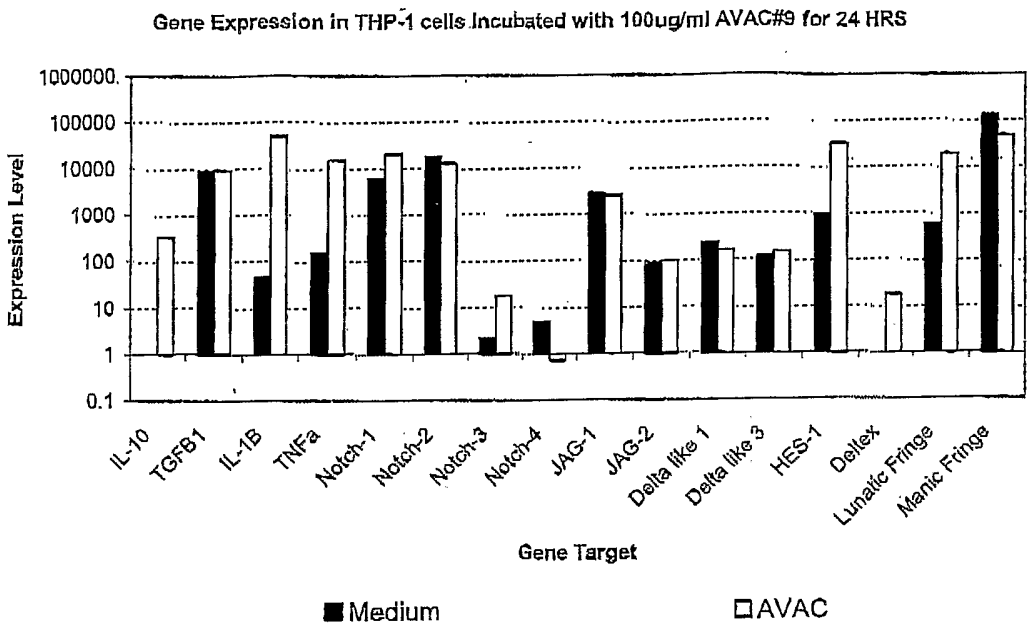

Gene Expression in THP-1 cells Incubated with 100ug/ml AVAC#9 for 24 HRS
Expression Level
1000000
100000
10000
1000
100
10
1
0.1
IL-10
TGFB1
IL-1B
TNFa
Notch-1
Notch-2
Notch-3
Notch-4
JAG-1
JAG-2
Delta like 1
Delta like 3
HES-1
Deltex
Lunatic Fringe
Manic Fringe
Gene Target
Medium
AVAC

Figure 15C

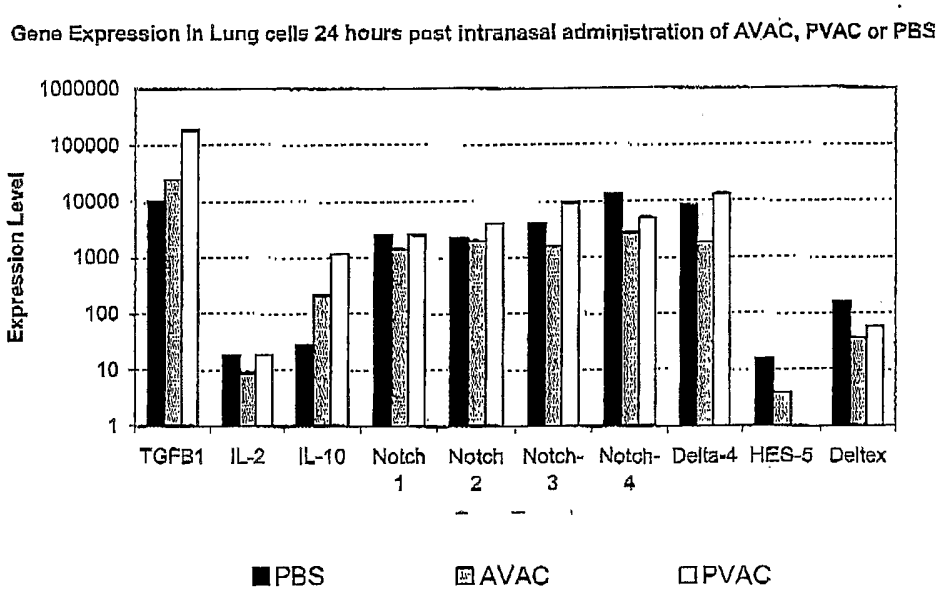

Gene Expression in Lung cells 24 hours post intranasal administration of AVAC, PVAC or PBS
Expression Level
1000000
100000
10000
1000
100
10
1
TGFB1
IL-2
IL-10
Notch 1
Notch 2
Notch-3
Notch-4
Delta-4
HES-5
Deltex
PBS
AVAC
PVAC

Figure 16

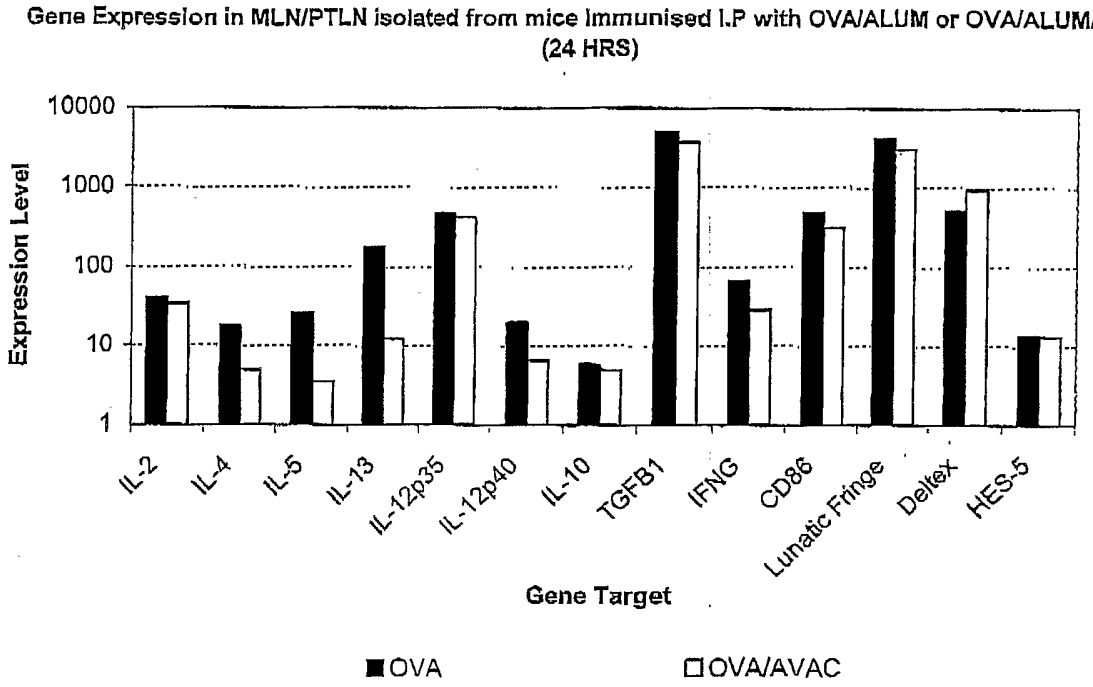

Gene Expression in MLN/PTLN isolated from mice immunised I.P with OVA/ALUM or OVA/ALUM/A
(24 HRS)
Expression Level
10000
1000
100
10
1
IL-2
IL-4
IL-5
IL-13
IL-12p35
IL-12p40
IL-10
TGFB1
IFNG
CD86
Lunatic Fringe
Deltex
HES-5
Gene Target
OVA
OVA/AVAC

Figure 17

Figure 18

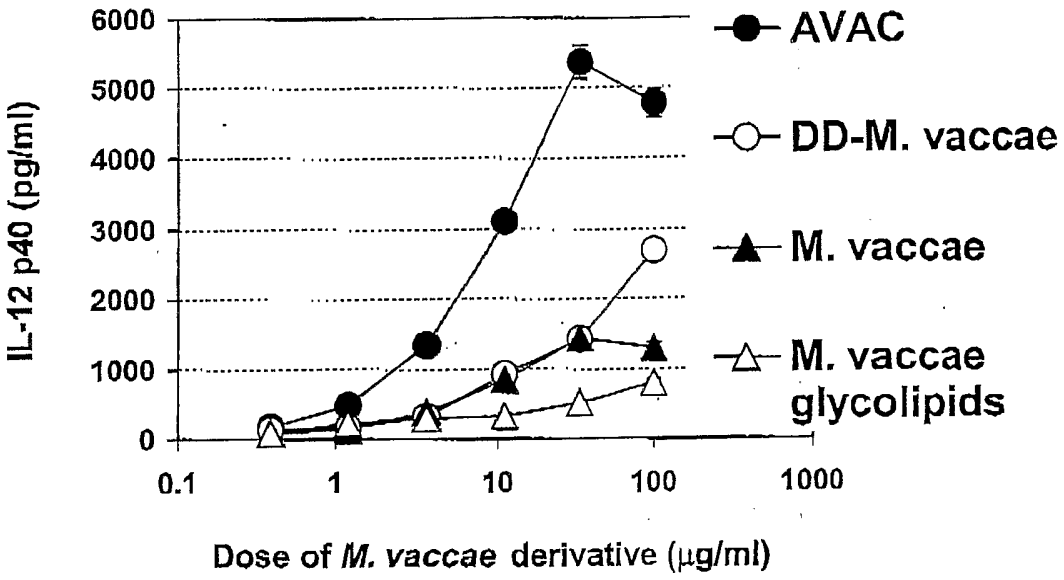
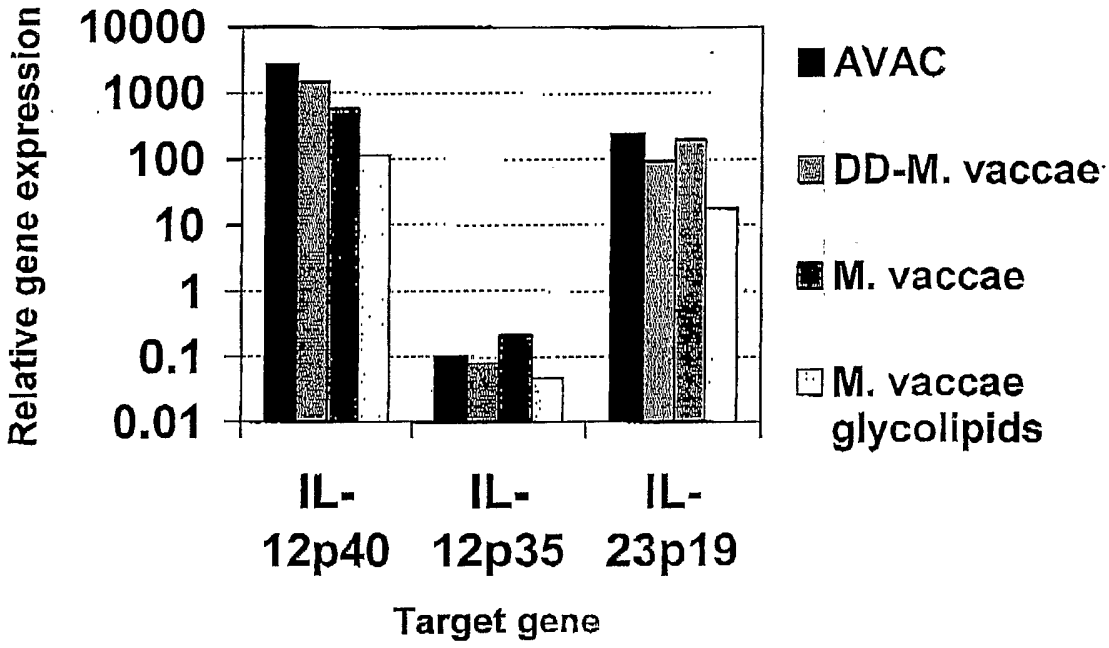

6000
5000
4000
3000
2000
1000
0
IL-12 p40 (pg/ml)
0.1
1
10
100
1000
Dose of M. vaccae derivative (μg/ml)
AVAC
DD-M. vaccae
M. vaccae
M. vaccae glycolipids

Figure 19


Relative gene expression
10000
1000
100
10
1
0.1
0.01
IL-12p40
IL-12p35
IL-23p19
Target gene
AVAC
DD-M. vaccae
M. vaccae
M. vaccae glycolipids

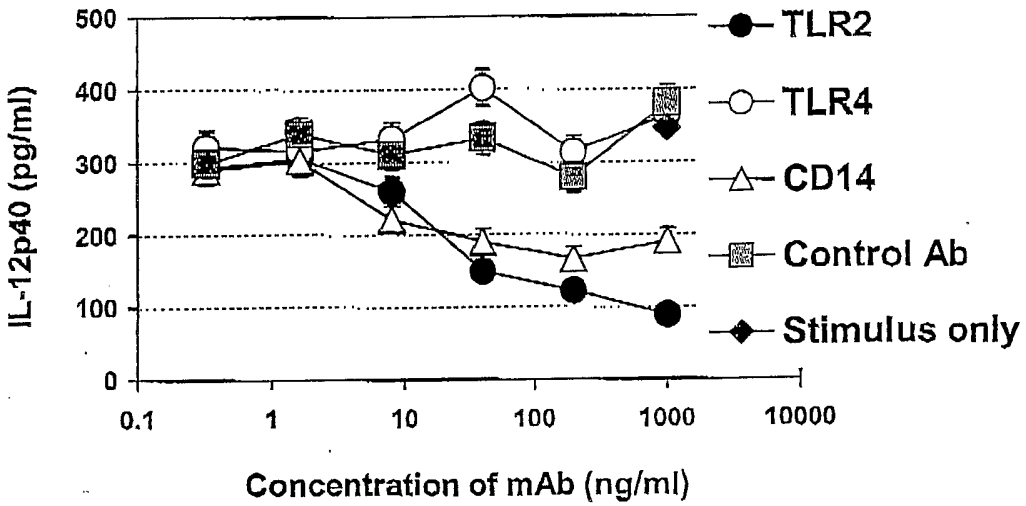

500
400
300
200
100
0
IL-12p40 (pg/ml)
0.1
1
10
100
1000
10000
Concentration of mAb (ng/ml)
TLR2
TLR4
CD14
Control Ab
Stimulus only

Figure 20A

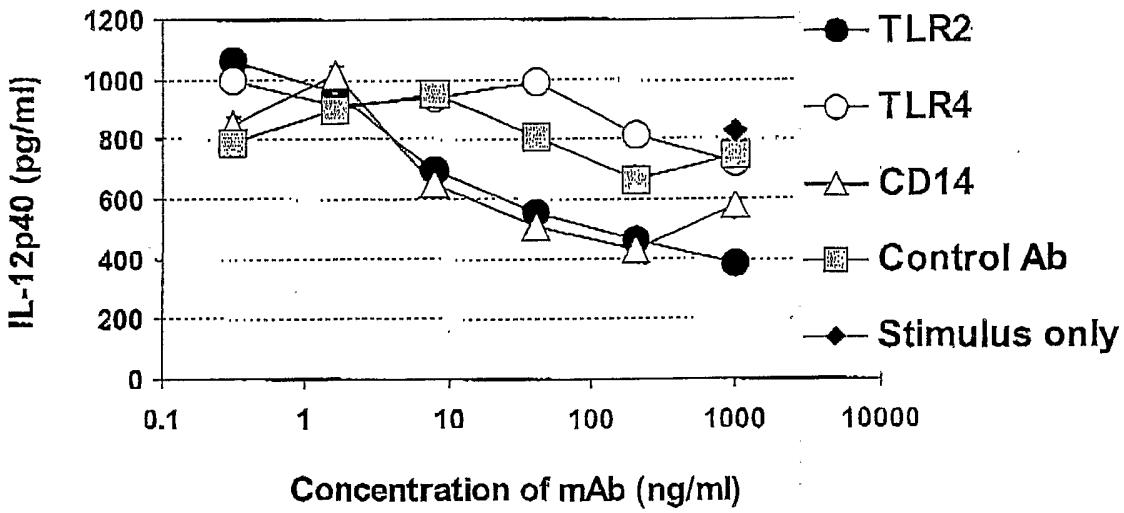

1200
1000
800
600
400
200
0
IL-12p40 (pg/ml)
0.1
1
10
100
1000
10000
Concentration of mAb (ng/ml)
TLR2
TLR4
CD14
Control Ab
Stimulus only

Figure 20B

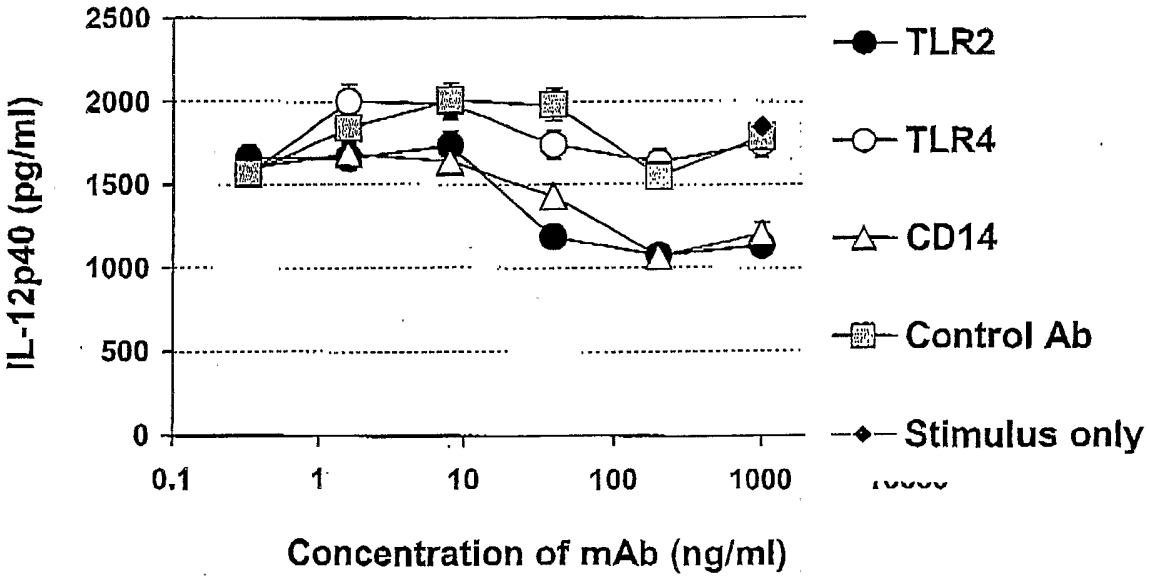
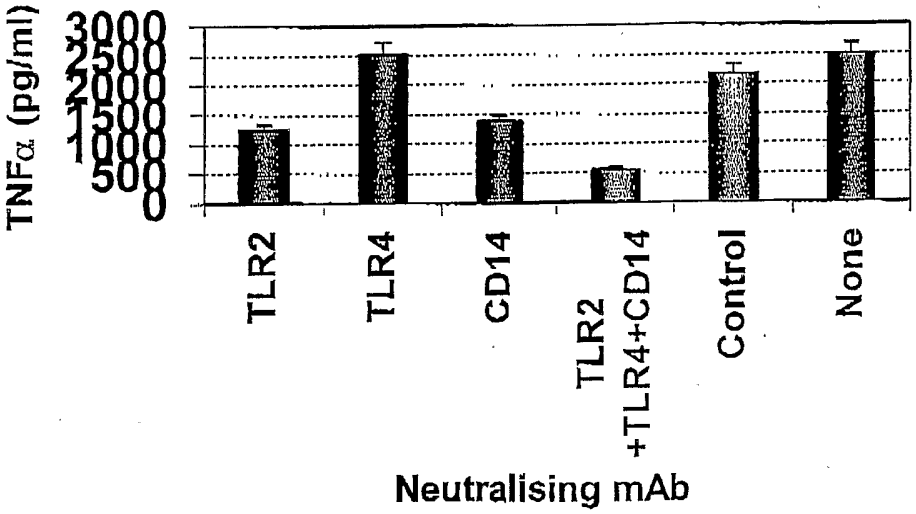

2500
2000
1500
1000
500
0
IL-12p40 (pg/ml)
0.1
1
10
100
1000
Concentration of mAb (ng/ml)
TLR2
TLR4
CD14
Control Ab
Stimulus only

Figure 20C

Figure 21A


TNFα (pg/ml)
3000
2500
2000
1500
1000
500
0
TLR2
TLR4
CD14
TLR2 +TLR4+CD14
Control
None
Neutralising mAb

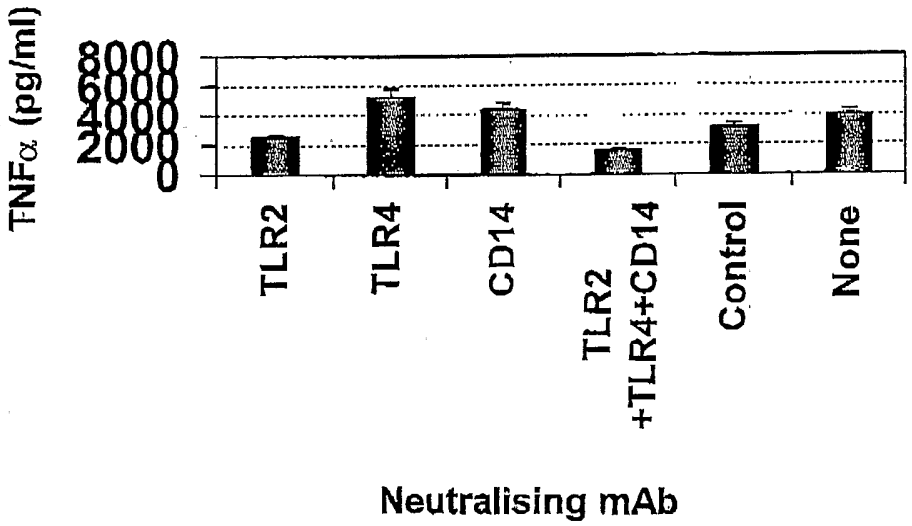

TNFα (pg/ml)
8000
6000
4000
2000
0
TLR2
TLR4
CD14
TLR2 +TLR4+CD14
Control
None
Neutralising mAb

Figure 21B

Figure 21C

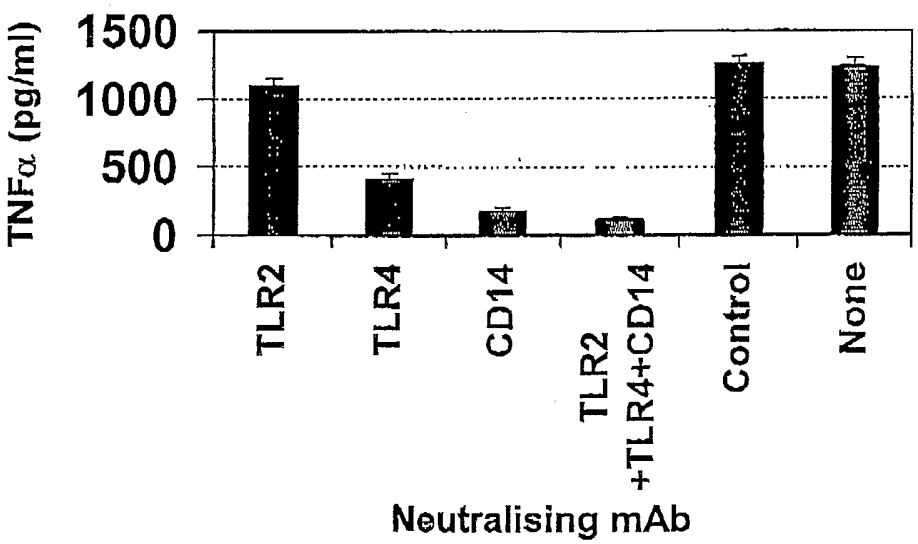

TNFα (pg/ml)
1500
1000
500
0
TLR2
TLR4
CD14
TLR2 +TLR4+CD14
Control
None
Neutralising mAb

Figure 22

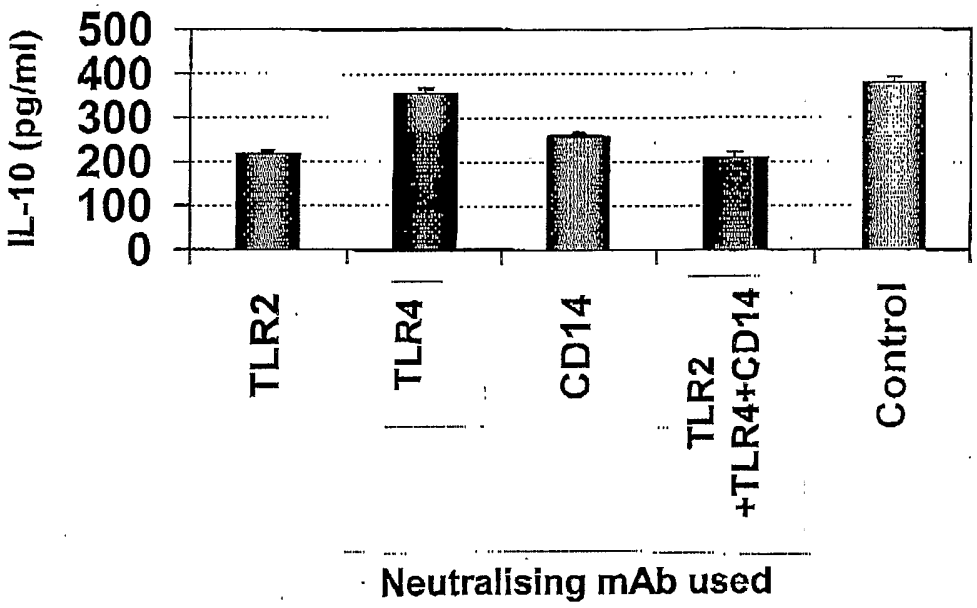
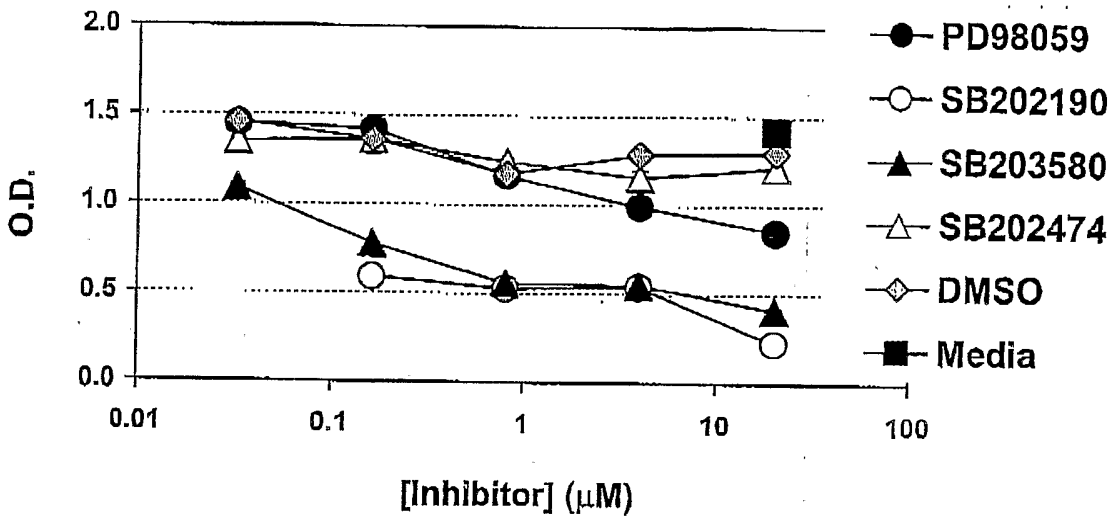

IL-10 (pg/ml)
500
400
300
200
100
0
TLR2
TLR4
CD14
TLR2 +TLR4+CD14
Control
Neutralising mAb used

Figure 23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1.5
1.0
0.5
0.0
O.D.
0.01
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1
10
100
[Inhibitor] (μM)
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SB203580
SB202474
DMSO
Media

SEQUENCE LISTING

<110> Watson, James D.
Tan, Paul L. J.
Abernethy, Nevin

<120> Compounds and Methods for the Modulation of Immune Responses

<130> 11000.1063U

<150> 60/308,446
<151> 2001-07-26

<160> 52

<170> FastSEQ for Windows Version 4.0

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gggtaccgca ggcagcgccg cagccgcgcc ggtcggaccg gggtgtgcgg cctacgtgca 180
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gctgaccaag atcctcacct accacgtcgt gcccggccag gccgcgcccg atcaggtggt 480
cggcgagcat gtgacggtgg agggggcgcc ggtcacggtg tccgggatgg ccgaccagct 540
caaggtcaac gacgcgtcgg tggtgtgcgg tggggtgcag accgccaacg cgacggtgta 600
tctgatcgac accgtgctga tgccgccggc agcgtagccg ggcggcacca cagaagaggg 660
tcccccgcac ccggcctccc ccg 683

<210> 2
<211> 808
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> misc_feature
<222> (1)...(808)
<223> n = A,T,C or G

<400> 2
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aatgatgaca actcgccgga agtcagccgc agtggcggga atcgctgcgg tggccatcct 180
cggtgcggcc gcatgttcga gtgaggacgg tgggagcacg gcctcgtcgg ccagcagcac 240
ggcctcctcc gcgatggagt ccgcgaccga cgagatgacc acgtcgtcgg cggccccttc 300
ggccgaccct gcggccaacc tgatcggctc cggctgcgcg gcctacgccg agcaggtccc 360
cgaaggtccc gggtcggtgg ccgggatggc agccgatccg gtgacggtgg cggcgtcgaa 420
caacccgatg ctgcagacgc tgtcccaggc gctgtccggc cagctcaatc cgcaggtcaa 480

tctcgtcgac accctcgacg gcggtgagtt caccgtgttc gcgccgaccg acgacgcgtt 540
cgccaagatc gatccggcca cgctggagac cctcaagacg gactccgaca tgctgaccaa 600
catcctgacc taccacgtcg tgcccggcca ggccgcgccc gatcaggtgg tcggcgagca 660
tgtgacggtg gaggggcgc cggtcacggt gtccgggatg gccgaccagc tcaaggtcaa 720
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caccgtgctg atgccgccgg cagcgtag 808

<210> 3
<211> 1211
<212> DNA
<213> Mycobacterium vaccae

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gcacgccgtt tcggcgtcgt ggctgtcgcg acagcgatga tgcctgcttt ggtgggcctg 120
gctggagggt cggcgaccgc cggagcattc tcccggccag gtctgccggt ggagtacctg 180
atggtgcctt cgccgtcgat ggggcgcgac atcaagatcc agttccagag cggtggcgag 240
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ttcctgaacc cctccgaggg ctggtggccg ttcctgatca acatctcgat gggtgacgcc 660
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tccagcgtga a 1211

<210> 4
<211> 485
<212> DNA
<213> Mycobacterium vaccae

<400> 4
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atcatgcccg tcggcgggca gtccagcttc tacagcgact ggtacagccc ggcctgcggt 120
aaggccggct gccagaccta caagtgggag acgttcctga cccaggagct gccggcctac 180
ctcgccgcca acaaggggt cgacccgaac cgcaacgcgg ccgtcggtct gtccatggcc 240
ggttcggcgg cgctgacgct ggcgatctac cacccgcagc agttccagta cgccgggtcg 300
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ctctc 485

<210> 5
<211> 1052
<212> DNA
<213> Mycobacterium vaccae

<400> 5

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ctgatcggct tcgccggggg ttcggcaacg gccggggcat tctcccggcc cggtcttcct 180
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ggctgggaca tcaacacccc tgcgttcgag tggttctacg agtccggctt gtcgacgatc 360
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gggcagaact acacctacaa gtgggagacg ttcctgaccc aggagctgcc gacgtggctg 480
gaggccaacc gcggagtgtc gcgcaccggc aacgcgttcg tcggcctgtc gatggcgggc 540
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caggccaccg cctagccacc caccccacac cc 1052

<210> 6
<211> 480
<212> DNA
<213> Mycobacterium vaccae

<400> 6
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aacgggcaga actacaccta caagtgggag acgttcctga cccaggagct gccgacgtgg 180
ctggaggcca accgcggagt gtcgcgcacc ggcaacgcgt tcgtcggcct gtcgatggcg 240
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aagcgcaacg acccgatggt caacatcaac cagctggtgg ccaacaacac ccggatctgg 480

<210> 7
<211> 795
<212> DNA
<213> Mycobacterium vaccae

<400> 7
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ccgaacgcgc actgcgacat cgccgtcgat ggccaggagg tgctcagcca gcacgacgac 480
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cgatggcacc ggcga 795

<210> 8
<211> 1125

<212> DNA
<213> Mycobacterium vaccae

<220>
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<222> (1)...(1125)
<223> n = A,T,C or G

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atcttcgcgc gcgggaccgg tgcggaaccc ggcctcgggt gggtcggtga tgcgttcgtc 180
aacgcgctgc ggcccaaggt cggtgagcag tcggtgggca cctacgcggt gaactacccg 240
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cccgatgccg ccccgcgtcg ccgaccacgt ggccgccgtt gtggtcttcg gaaatccgtt 480
gcgcgacatc cgtggtggcg gtccgctgcc gcagatgagc ggcacctacg ggccgaagtc 540
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ggtaacgctg gaggacgccg gaccgctgcg cgaaggcgac gcagtgcgtt tcaccgcatc 780
gggcggccag cgggtgaccg ccaccgcgcc cgcggagatc ctcgtctggg agatgcatgc 840
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gggcgcggac cgcccggccg cggctggccg cgtgggcccc ggacg 1125

<210> 9
<211> 650
<212> DNA
<213> Mycobacterium vaccae

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<210> 10
<211> 501
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> misc_feature
<222> (1)...(501)
<223> n = A,T,C or G

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<210> 11
<211> 554
<212> DNA
<213> Mycobacterium vaccae

<400> 11
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tcatggccgg ctga 554

<210> 12
<211> 1518
<212> DNA
<213> Mycobacterium vaccae

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catcagtaca gcgcgctttc ctgcgcggat tctattgtcg agtccggggt gtgacgaagg 180
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cacatcaccg ctgcagaacc tgcagaacag acggcggatt ccgcggcacc gcccaagggc 360
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ttccagcact acgcgctgtt cccgcacatg acggtctggg acaacgtcgc gtacggcccg 660
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acgcttcccg attgccga 1518

<210> 13
<211> 1111
<212> DNA
<213> Mycobacterium vaccae

<400> 13
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gcccaatcgc aagaatctgc gtcaggacct gttggactcg agcatcgacg agggccgcaa 360
gttcaccgcg ccgtacatga ccggcatggt cggtctcgcc tacaacaagg cagccaccgg 420
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<210> 14
<211> 1626
<212> DNA
<213> Mycobacterium vaccae

<400> 14
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aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag 180
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gagcagattt ctgccaccgc ggcgatttcc gccggcgaca cccagatcgg cgagctcatc 480
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<211> 743
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<213> Mycobacterium vaccae

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<212> DNA
<213> Mycobacterium vaccae

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<211> 898
<212> DNA
<213> Mycobacterium vaccae

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<211> 2013
<212> DNA
<213> Mycobacterium vaccae

<400> 21
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<213> Mycobacterium vaccae

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<213> Mycobacterium vaccae

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<213> Mycobacterium vaccae

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<212> DNA
<213> Mycobacterium vaccae

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<400> 27
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Ala Gly Phe Ala Met Thr Ala Ala Val Gly Leu Ser Leu Gly Thr Ala

        35                  40                  45
Gly Ser Ala Ala Ala Ala Pro Val Gly Pro Gly Cys Ala Ala Tyr Val
    50                  55                  60
Gln Gln Val Pro Asp Gly Pro Gly Ser Val Gln Gly Met Ala Ser Ser
65                  70                  75                  80
Pro Val Ala Thr Ala Ala Ala Asp Asn Pro Leu Leu Thr Thr Leu Ser
                85                  90                  95
Gln Ala Ile Ser Gly Gln Leu Asn Pro Asn Val Asn Leu Val Asp Thr
            100                 105                 110
Phe Asn Gly Gly Gln Phe Thr Val Phe Ala Pro Thr Asn Asp Ala Phe
        115                 120                 125
Ala Lys Ile Asp Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp
    130                 135                 140
Leu Leu Thr Lys Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala
145                 150                 155                 160
Pro Asp Gln Val Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val
                165                 170                 175
Thr Val Ser Gly Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val
            180                 185                 190
Val Cys Gly Gly Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp
        195                 200                 205
Thr Val Leu Met Pro Pro Ala Ala Pro Gly Gly Thr Thr Glu Glu Gly
    210                 215                 220
Pro Pro His Pro Ala Ser Pro
225                 230

<210> 28
<211> 228
<212> PRT
<213> Mycobacterium vaccae

<400> 28
Met Met Thr Thr Arg Arg Lys Ser Ala Ala Val Ala Gly Ile Ala Ala
 1               5                  10                  15
Val Ala Ile Leu Gly Ala Ala Ala Cys Ser Ser Glu Asp Gly Gly Ser
            20                  25                  30
```

```
Thr Ala Ser Ser Ala Ser Ser Thr Ala Ser Ser Ala Met Glu Ser Ala
        35                  40                  45
Thr Asp Glu Met Thr Thr Ser Ser Ala Ala Pro Ser Ala Asp Pro Ala
    50                  55                  60
Ala Asn Leu Ile Gly Ser Gly Cys Ala Ala Tyr Ala Glu Gln Val Pro
65                  70                  75                  80
Glu Gly Pro Gly Ser Val Ala Gly Met Ala Ala Asp Pro Val Thr Val
                85                  90                  95
Ala Ala Ser Asn Asn Pro Met Leu Gln Thr Leu Ser Gln Ala Leu Ser
            100                 105                 110
Gly Gln Leu Asn Pro Gln Val Asn Leu Val Asp Thr Leu Asp Gly Gly
        115                 120                 125
Glu Phe Thr Val Phe Ala Pro Thr Asp Asp Ala Phe Ala Lys Ile Asp
    130                 135                 140
Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp Met Leu Thr Asn
145                 150                 155                 160
Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala Pro Asp Gln Val
                165                 170                 175
Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val Thr Val Ser Gly
            180                 185                 190
Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val Val Cys Gly Gly
        195                 200                 205
Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp Thr Val Leu Met
    210                 215                 220
Pro Pro Ala Ala
225
```

<210> 29
<211> 326
<212> PRT
<213> Mycobacterium vaccae

<400> 29

```
Met Arg Leu Leu Asp Arg Ile Arg Gly Pro Trp Ala Arg Arg Phe Gly
 1               5                  10                  15
Val Val Ala Val Ala Thr Ala Met Met Pro Ala Leu Val Gly Leu Ala
            20                  25                  30
Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
        35                  40                  45
Glu Tyr Leu Met Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Ile
    50                  55                  60
Gln Phe Gln Ser Gly Gly Glu Asn Ser Pro Ala Leu Tyr Leu Leu Asp
65                  70                  75                  80
Gly Leu Arg Ala Gln Glu Asp Phe Asn Gly Trp Asp Ile Asn Thr Gln
                85                  90                  95
Ala Phe Glu Trp Phe Leu Asp Ser Gly Ile Ser Val Val Met Pro Val
            100                 105                 110
Gly Gly Gln Ser Ser Phe Tyr Thr Asp Trp Tyr Ala Pro Ala Arg Asn
        115                 120                 125
Lys Gly Pro Thr Val Thr Tyr Lys Trp Glu Thr Phe Leu Thr Gln Glu
    130                 135                 140
Leu Pro Gly Trp Leu Gln Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
145                 150                 155                 160
Gly Pro Val Gly Leu Ser Met Ala Gly Ser Ala Ala Leu Asn Leu Ala
                165                 170                 175
Thr Trp His Pro Glu Gln Phe Ile Tyr Ala Gly Ser Met Ser Gly Phe
```

```
            180                 185                 190
Leu Asn Pro Ser Glu Gly Trp Trp Pro Phe Leu Ile Asn Ile Ser Met
        195                 200                 205
Gly Asp Ala Gly Gly Phe Lys Ala Asp Asp Met Trp Gly Lys Thr Glu
    210                 215                 220
Gly Ile Pro Thr Ala Val Gly Gln Arg Asn Asp Pro Met Leu Asn Ile
225                 230                 235                 240
Pro Thr Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly Asn
                245                 250                 255
Gly Gln Pro Thr Glu Leu Gly Gly Gly Asp Leu Pro Ala Thr Phe Leu
            260                 265                 270
Glu Gly Leu Thr Ile Arg Thr Asn Glu Thr Phe Arg Asp Asn Tyr Ile
        275                 280                 285
Ala Ala Gly Gly His Asn Gly Val Phe Asn Phe Pro Ala Asn Gly Thr
    290                 295                 300
His Asn Trp Ala Tyr Trp Gly Arg Glu Leu Gln Ala Met Lys Pro Asp
305                 310                 315                 320
Leu Gln Ala His Leu Leu
                325
```

<210> 30
<211> 161
<212> PRT
<213> Mycobacterium vaccae

<400> 30

```
Ser Gly Trp Asp Ile Asn Thr Ala Ala Phe Glu Trp Tyr Val Asp Ser
 1               5                  10                  15
Gly Leu Ala Val Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
            20                  25                  30
Asp Trp Tyr Ser Pro Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys
        35                  40                  45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Ala Tyr Leu Ala Ala Asn
    50                  55                  60
Lys Gly Val Asp Pro Asn Arg Asn Ala Ala Val Gly Leu Ser Met Ala
65                  70                  75                  80
Gly Ser Ala Ala Leu Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Gln
                85                  90                  95
Tyr Ala Gly Ser Leu Ser Gly Tyr Leu Asn Pro Ser Glu Gly Trp Trp
            100                 105                 110
Pro Met Leu Ile Asn Ile Ser Met Gly Asp Ala Gly Gly Tyr Lys Ala
        115                 120                 125
Asn Asp Met Trp Gly Arg Thr Glu Asp Pro Ser Ser Ala Trp Lys Arg
    130                 135                 140
Asn Asp Pro Met Val Asn Ile Gly Lys Leu Val Ala Asn Asn Thr Pro
145                 150                 155                 160
Leu
```

<210> 31
<211> 334
<212> PRT
<213> Mycobacterium vaccae

<400> 31

```
Met Lys Phe Thr Glu Lys Trp Arg Gly Ser Ala Lys Ala Ala Met His
 1               5                  10                  15
```

```
Arg Val Gly Val Ala Asp Met Ala Ala Val Ala Leu Pro Gly Leu Ile
            20                  25                  30
Gly Phe Ala Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly
        35                  40                  45
Leu Pro Val Glu Tyr Leu Asp Val Phe Ser Pro Ser Met Gly Arg Asp
    50                  55                  60
Ile Arg Val Gln Phe Gln Gly Gly Gly Thr His Ala Val Tyr Leu Leu
65                  70                  75                  80
Asp Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr
                85                  90                  95
Pro Ala Phe Glu Trp Phe Tyr Glu Ser Gly Leu Ser Thr Ile Met Pro
            100                 105                 110
Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Gln Pro Ser Arg
        115                 120                 125
Gly Asn Gly Gln Asn Tyr Thr Tyr Lys Trp Glu Thr Phe Leu Thr Gln
    130                 135                 140
Glu Leu Pro Thr Trp Leu Glu Ala Asn Arg Gly Val Ser Arg Thr Gly
145                 150                 155                 160
Asn Ala Phe Val Gly Leu Ser Met Ala Gly Ser Ala Ala Leu Thr Tyr
                165                 170                 175
Ala Ile His His Pro Gln Gln Phe Ile Tyr Ala Ser Ser Leu Ser Gly
            180                 185                 190
Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Met Leu Ile Gly Leu Ala
        195                 200                 205
Met Asn Asp Ala Gly Gly Phe Asn Ala Glu Ser Met Trp Gly Pro Ser
    210                 215                 220
Ser Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Asn Ile Asn Gln
225                 230                 235                 240
Leu Val Ala Asn Asn Thr Arg Ile Trp Ile Tyr Cys Gly Thr Gly Thr
                245                 250                 255
Pro Ser Glu Leu Asp Thr Gly Thr Pro Gly Gln Asn Leu Met Ala Ala
            260                 265                 270
Gln Phe Leu Glu Gly Phe Thr Leu Arg Thr Asn Ile Ala Phe Arg Asp
        275                 280                 285
Asn Tyr Ile Ala Ala Gly Gly Thr Asn Gly Val Phe Asn Phe Pro Ala
    290                 295                 300
Ser Gly Thr His Ser Trp Gly Tyr Trp Gly Gln Gln Leu Gln Gln Met
305                 310                 315                 320
Lys Pro Asp Ile Gln Arg Val Leu Gly Ala Gln Ala Thr Ala
                325                 330
```

<210> 32
<211> 161
<212> PRT
<213> Mycobacterium vaccae

```
<400> 32
Asn Gly Trp Asp Ile Asn Thr Pro Ala Phe Glu Trp Phe Tyr Glu Ser
 1               5                  10                  15
Gly Leu Ser Thr Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
            20                  25                  30
Asp Trp Tyr Gln Pro Ser Arg Gly Asn Gly Gln Asn Tyr Thr Tyr Lys
        35                  40                  45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Thr Trp Leu Glu Ala Asn
    50                  55                  60
Arg Gly Val Ser Arg Thr Gly Asn Ala Phe Val Gly Leu Ser Met Ala
65                  70                  75                  80
```

```
Gly Ser Ala Ala Leu Thr Tyr Ala Ile His His Pro Gln Gln Phe Ile
                85                  90                  95
Tyr Ala Ser Ser Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp
            100                 105                 110
Pro Met Leu Ile Gly Leu Ala Met Asn Asp Ala Gly Gly Phe Asn Ala
        115                 120                 125
Glu Ser Met Trp Gly Pro Ser Ser Asp Pro Ala Trp Lys Arg Asn Asp
    130                 135                 140
Pro Met Val Asn Ile Asn Gln Leu Val Ala Asn Asn Thr Arg Ile Trp
145                 150                 155                 160
Ile
```

<210> 33
<211> 142
<212> PRT
<213> Mycobacterium vaccae

<400> 33

```
Met Arg Thr Ala Thr Thr Lys Leu Gly Ala Ala Leu Gly Ala Ala Ala
 1               5                  10                  15
Leu Val Ala Ala Thr Gly Met Val Ser Ala Ala Thr Ala Asn Ala Gln
            20                  25                  30
Glu Gly His Gln Val Arg Tyr Thr Leu Thr Ser Ala Gly Ala Tyr Glu
        35                  40                  45
Phe Asp Leu Phe Tyr Leu Thr Thr Gln Pro Pro Ser Met Gln Ala Phe
    50                  55                  60
Asn Ala Asp Ala Tyr Ala Phe Ala Lys Arg Glu Lys Val Ser Leu Ala
65                  70                  75                  80
Pro Gly Val Pro Trp Val Phe Glu Thr Thr Met Ala Asp Pro Asn Trp
                85                  90                  95
Ala Ile Leu Gln Val Ser Ser Thr Thr Arg Gly Gly Gln Ala Ala Pro
            100                 105                 110
Asn Ala His Cys Asp Ile Ala Val Asp Gly Gln Glu Val Leu Ser Gln
        115                 120                 125
His Asp Asp Pro Tyr Asn Val Arg Cys Gln Leu Gly Gln Trp
    130                 135                 140
```

<210> 34
<211> 285
<212> PRT
<213> Mycobacterium vaccae

<400> 34

```
Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
 1               5                  10                  15
Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
            20                  25                  30
Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
        35                  40                  45
Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
    50                  55                  60
Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
65                  70                  75                  80
Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
                85                  90                  95
Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
```

```
            100                 105                 110
Val Leu Gly Gly Met Ser Gln Gly Ala Gly Val Ile Asp Leu Ile Thr
        115                 120                 125
Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
    130                 135                 140
Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
145                 150                 155                 160
Arg Asp Ile Arg Gly Gly Gly Pro Leu Pro Gln Met Ser Gly Thr Tyr
                165                 170                 175
Gly Pro Lys Ser Ile Asp Leu Cys Ala Leu Asp Asp Pro Phe Cys Ser
            180                 185                 190
Pro Gly Phe Asn Leu Pro Ala His Phe Ala Tyr Ala Asp Asn Gly Met
        195                 200                 205
Val Glu Glu Ala Ala Asn Phe Ala Arg Leu Glu Pro Gly Gln Ser Val
    210                 215                 220
Glu Leu Pro Glu Ala Pro Tyr Leu His Leu Phe Val Pro Arg Gly Glu
225                 230                 235                 240
Val Thr Leu Glu Asp Ala Gly Pro Leu Arg Glu Gly Asp Ala Val Arg
                245                 250                 255
Phe Thr Ala Ser Gly Gly Gln Arg Val Thr Ala Thr Ala Pro Ala Glu
            260                 265                 270
Ile Leu Val Trp Glu Met His Ala Gly Leu Gly Ala Ala
        275                 280                 285
```

<210> 35
<211> 159
<212> PRT
<213> Mycobacterium vaccae

<400> 35

```
Met Thr Ala Gly Ala Ala Ala Ala Ala Thr Leu Gly Ala Ala Ala Val
 1               5                  10                  15
Gly Val Thr Ser Ile Ala Val Gly Ala Gly Val Ala Gly Ala Ser Pro
            20                  25                  30
Ala Val Leu Asn Ala Pro Leu Leu Ser Ala Pro Ala Pro Asp Leu Gln
        35                  40                  45
Gly Pro Leu Val Ser Thr Leu Ser Ala Leu Ser Gly Pro Gly Ser Phe
    50                  55                  60
Ala Gly Ala Lys Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu
65                  70                  75                  80
Ala Arg Val Ala Asp Ser Gly Tyr Ser Asn Ala Ala Ala Lys Gly Tyr
                85                  90                  95
Phe Pro Leu Ser Phe Thr Val Ala Gly Ile Asp Gln Asn Gly Pro Ile
            100                 105                 110
Val Thr Ala Asn Val Thr Ala Ala Ala Pro Thr Gly Ala Val Ala Thr
        115                 120                 125
Gln Pro Leu Thr Phe Ile Ala Gly Pro Ser Pro Thr Gly Trp Gln Leu
    130                 135                 140
Ser Lys Gln Ser Ala Leu Ala Leu Met Ser Ala Val Ile Ala Ala
145                 150                 155
```

<210> 36
<211> 166
<212> PRT
<213> Mycobacterium vaccae

<400> 36

```
Met Pro Val Arg Arg Ala Arg Ser Ala Leu Ala Ser Val Thr Phe Val
 1               5                  10                  15
Ala Ala Ala Cys Val Gly Ala Glu Gly Thr Ala Leu Ala Ala Thr Pro
            20                  25                  30
Asp Trp Ser Gly Arg Tyr Thr Val Val Thr Phe Ala Ser Asp Lys Leu
        35                  40                  45
Gly Thr Ser Val Ala Ala Arg Gln Pro Glu Pro Asp Phe Ser Gly Gln
    50                  55                  60
Tyr Thr Phe Ser Thr Ser Cys Val Gly Thr Cys Val Ala Thr Ala Ser
65                  70                  75                  80
Asp Gly Pro Ala Pro Ser Asn Pro Thr Ile Pro Gln Pro Ala Arg Tyr
                85                  90                  95
Thr Trp Asp Gly Arg Gln Trp Val Phe Asn Tyr Asn Trp Gln Trp Glu
            100                 105                 110
Cys Phe Arg Gly Ala Asp Val Pro Arg Glu Tyr Ala Ala Ala Arg Ser
        115                 120                 125
Leu Val Phe Tyr Ala Pro Thr Ala Asp Gly Ser Met Phe Gly Thr Trp
    130                 135                 140
Arg Thr Asp Ile Leu Asp Gly Leu Cys Lys Gly Thr Val Ile Met Pro
145                 150                 155                 160
Val Ala Ala Tyr Pro Ala
                165
```

<210> 37
<211> 136
<212> PRT
<213> Mycobacterium vaccae

<400> 37

```
Met Lys Phe Thr Gly Met Thr Val Arg Ala Ser Arg Arg Ala Leu Ala
 1               5                  10                  15
Gly Val Gly Ala Ala Cys Leu Phe Gly Gly Val Ala Ala Ala Thr Val
            20                  25                  30
Ala Ala Gln Met Ala Gly Ala Gln Pro Ala Glu Cys Asn Ala Ser Ser
        35                  40                  45
Leu Thr Gly Thr Val Ser Ser Val Thr Gly Gln Ala Arg Gln Tyr Leu
    50                  55                  60
Asp Thr His Pro Gly Ala Asn Gln Ala Val Thr Ala Ala Met Asn Gln
65                  70                  75                  80
Pro Arg Pro Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr Ala Asn
                85                  90                  95
Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile Gly Asp
            100                 105                 110
Ala Gln Arg Asn Cys Asn Ile Thr Val Leu Pro Val Glu Leu Gln Thr
        115                 120                 125
Ala Tyr Asp Thr Phe Met Ala Gly
    130                 135
```

<210> 38
<211> 376
<212> PRT
<213> Mycobacterium vaccae

<400> 38

```
Val Ile Glu Ile Asp His Val Thr Lys Arg Phe Gly Asp Tyr Leu Ala
 1               5                  10                  15
Val Ala Asp Ala Asp Phe Ser Ile Ala Pro Gly Glu Phe Phe Ser Met
```

```
            20                  25                  30
Leu Gly Pro Ser Gly Cys Gly Lys Thr Thr Thr Leu Arg Met Ile Ala
        35                  40                  45
Gly Phe Glu Thr Pro Thr Glu Gly Ala Ile Arg Leu Glu Gly Ala Asp
    50                  55                  60
Val Ser Arg Thr Pro Pro Asn Lys Arg Asn Val Asn Thr Val Phe Gln
65                  70                  75                  80
His Tyr Ala Leu Phe Pro His Met Thr Val Trp Asp Asn Val Ala Tyr
                85                  90                  95
Gly Pro Arg Ser Lys Lys Leu Gly Lys Gly Glu Val Arg Lys Arg Val
            100                 105                 110
Asp Glu Leu Leu Glu Ile Val Arg Leu Thr Glu Phe Ala Glu Arg Arg
        115                 120                 125
Pro Ala Gln Leu Ser Gly Gly Gln Gln Gln Arg Val Ala Leu Ala Arg
    130                 135                 140
Ala Leu Val Asn Tyr Pro Ser Ala Leu Leu Leu Asp Glu Pro Leu Gly
145                 150                 155                 160
Ala Leu Asp Leu Lys Leu Arg His Val Met Gln Phe Glu Leu Lys Arg
                165                 170                 175
Ile Gln Arg Glu Val Gly Ile Thr Phe Ile Tyr Val Thr His Asp Gln
            180                 185                 190
Glu Glu Ala Leu Thr Met Ser Asp Arg Ile Ala Val Met Asn Ala Gly
        195                 200                 205
Asn Val Glu Gln Ile Gly Ser Pro Thr Glu Ile Tyr Asp Arg Pro Ala
    210                 215                 220
Thr Val Phe Val Ala Ser Phe Ile Gly Gln Ala Asn Leu Trp Ala Gly
225                 230                 235                 240
Arg Cys Thr Gly Arg Ser Asn Arg Asp Tyr Val Glu Ile Asp Val Leu
                245                 250                 255
Gly Ser Thr Leu Lys Ala Arg Pro Gly Glu Thr Thr Ile Glu Pro Gly
            260                 265                 270
Gly His Ala Thr Leu Met Val Arg Pro Glu Arg Ile Arg Val Thr Pro
        275                 280                 285
Gly Ser Gln Asp Ala Pro Thr Gly Asp Val Ala Cys Val Arg Ala Thr
    290                 295                 300
Val Thr Asp Leu Thr Phe Gln Gly Pro Val Val Arg Leu Ser Leu Ala
305                 310                 315                 320
Ala Pro Asp Asp Ser Thr Val Ile Ala His Val Gly Pro Glu Gln Asp
                325                 330                 335
Leu Pro Leu Leu Arg Pro Gly Asp Asp Val Tyr Val Ser Trp Ala Pro
            340                 345                 350
Glu Ala Ser Leu Val Leu Pro Gly Asp Asp Ile Pro Thr Thr Glu Asp
        355                 360                 365
Leu Glu Glu Met Leu Asp Asp Ser
    370                 375
```

<210> 39
<211> 348
<212> PRT
<213> Mycobacterium vaccae

<400> 39

```
Ser Asp Ser Gly Thr Ser Ser Thr Thr Ser Gln Asp Ser Gly Pro Ala
 1               5                  10                  15
Ser Gly Ala Leu Arg Val Ser Asn Trp Pro Leu Tyr Met Ala Asp Gly
            20                  25                  30
Phe Ile Ala Ala Phe Gln Thr Ala Ser Gly Ile Thr Val Asp Tyr Lys
```

```
        35                  40                  45
Glu Asp Phe Asn Asp Asn Glu Gln Trp Phe Ala Lys Val Lys Glu Pro
    50                  55                  60
Leu Ser Arg Lys Gln Asp Ile Gly Ala Asp Leu Val Ile Pro Thr Glu
65                  70                  75                  80
Phe Met Ala Ala Arg Val Lys Gly Leu Gly Trp Leu Asn Glu Ile Ser
                85                  90                  95
Glu Ala Gly Val Pro Asn Arg Lys Asn Leu Arg Gln Asp Leu Leu Asp
            100                 105                 110
Ser Ser Ile Asp Glu Gly Arg Lys Phe Thr Ala Pro Tyr Met Thr Gly
        115                 120                 125
Met Val Gly Leu Ala Tyr Asn Lys Ala Ala Thr Gly Arg Asp Ile Arg
    130                 135                 140
Thr Ile Asp Asp Leu Trp Asp Pro Ala Phe Lys Gly Arg Val Ser Leu
145                 150                 155                 160
Phe Ser Asp Val Gln Asp Gly Leu Gly Met Ile Met Leu Ser Gln Gly
                165                 170                 175
Asn Ser Pro Glu Asn Pro Thr Thr Glu Ser Ile Gln Gln Ala Val Asp
            180                 185                 190
Leu Val Arg Glu Gln Asn Asp Arg Gly Gln Ile Arg Arg Phe Thr Gly
        195                 200                 205
Asn Asp Tyr Ala Asp Asp Leu Ala Ala Gly Asn Ile Ala Ile Ala Gln
    210                 215                 220
Ala Tyr Ser Gly Asp Val Val Gln Leu Gln Ala Asp Asn Pro Asp Leu
225                 230                 235                 240
Gln Phe Ile Val Pro Glu Ser Gly Gly Asp Trp Phe Val Asp Thr Met
                245                 250                 255
Val Ile Pro Tyr Thr Thr Gln Asn Gln Lys Ala Ala Glu Ala Trp Ile
            260                 265                 270
Asp Tyr Ile Tyr Asp Arg Ala Asn Tyr Ala Lys Leu Val Ala Phe Thr
        275                 280                 285
Gln Phe Val Pro Ala Leu Ser Asp Met Thr Asp Glu Leu Ala Lys Val
    290                 295                 300
Asp Pro Ala Ser Ala Glu Asn Pro Leu Ile Asn Pro Ser Ala Glu Val
305                 310                 315                 320
Gln Ala Asn Leu Lys Ser Trp Ala Ala Leu Thr Asp Glu Gln Thr Gln
                325                 330                 335
Glu Phe Asn Thr Ala Tyr Ala Ala Val Thr Gly Gly
            340                 345
```

<210> 40
<211> 541
<212> PRT
<213> Mycobacterium vaccae

<400> 40

```
Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1               5                  10                  15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
            20                  25                  30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
        35                  40                  45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
    50                  55                  60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65                  70                  75                  80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
```

```
                85                  90                  95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
            100                 105                 110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
        115                 120                 125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
    130                 135                 140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
145                 150                 155                 160
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
                165                 170                 175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
            180                 185                 190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
        195                 200                 205
Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys
    210                 215                 220
Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gln
225                 230                 235                 240
Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
                245                 250                 255
Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
            260                 265                 270
Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
        275                 280                 285
Asp Met Ala Ile Leu Thr Gly Gly Gln Val Val Ser Glu Arg Val Gly
    290                 295                 300
Leu Ser Leu Glu Thr Ala Asp Val Ser Leu Leu Gly Gln Ala Arg Lys
305                 310                 315                 320
Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ser Gly Asp
                325                 330                 335
Ser Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Ala Glu Ile Glu
            340                 345                 350
Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
        355                 360                 365
Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
    370                 375                 380
Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
385                 390                 395                 400
Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Ala
                405                 410                 415
Leu Leu Gln Ser Ala Pro Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp
            420                 425                 430
Glu Ala Thr Gly Ala Asn Ile Val Arg Val Ala Leu Ser Ala Pro Leu
        435                 440                 445
Lys Gln Ile Ala Phe Asn Gly Gly Leu Glu Pro Gly Val Val Ala Glu
    450                 455                 460
Lys Val Ser Asn Leu Pro Ala Gly His Gly Leu Asn Ala Ala Thr Gly
465                 470                 475                 480
Glu Tyr Glu Asp Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val
                485                 490                 495
Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu
            500                 505                 510
Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro
        515                 520                 525
Ala Gly Asp Pro Thr Gly Gly Met Gly Gly Met Asp Phe
    530                 535                 540
```

<210> 41
<211> 215
<212> PRT
<213> Mycobacterium vaccae

<400> 41
Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
1 5 10 15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
20 25 30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
35 40 45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
50 55 60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65 70 75 80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
85 90 95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
100 105 110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
115 120 125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
130 135 140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
145 150 155 160
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
165 170 175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
180 185 190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
195 200 205
Gln Glu Ala Val Leu Glu Asp
210 215

<210> 42
<211> 327
<212> PRT
<213> Mycobacterium vaccae

<400> 42
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1 5 10 15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
20 25 30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
35 40 45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
50 55 60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
65 70 75 80
Gly Gly Gln Val Val Ser Glu Arg Val Gly Leu Ser Leu Glu Thr Ala
85 90 95
Asp Val Ser Leu Leu Gly Gln Ala Arg Lys Val Val Val Thr Lys Asp
100 105 110
Glu Thr Thr Ile Val Glu Gly Ser Gly Asp Ser Asp Ala Ile Ala Gly

```
        115                 120                 125
Arg Val Ala Gln Ile Arg Ala Glu Ile Glu Asn Ser Asp Ser Asp Tyr
    130                 135                 140
Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly Val
145                 150                 155                 160
Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu Arg
                165                 170                 175
Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val Glu
            180                 185                 190
Glu Gly Ile Val Ala Gly Gly Gly Val Ala Leu Leu Gln Ser Ala Pro
        195                 200                 205
Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp Glu Ala Thr Gly Ala Asn
    210                 215                 220
Ile Val Arg Val Ala Leu Ser Ala Pro Leu Lys Gln Ile Ala Phe Asn
225                 230                 235                 240
Gly Gly Leu Glu Pro Gly Val Val Ala Glu Lys Val Ser Asn Leu Pro
                245                 250                 255
Ala Gly His Gly Leu Asn Ala Ala Thr Gly Glu Tyr Glu Asp Leu Leu
            260                 265                 270
Lys Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala Leu Gln
        275                 280                 285
Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu Thr Thr Glu Ala Val Val
    290                 295                 300
Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro Ala Gly Asp Pro Thr Gly
305                 310                 315                 320
Gly Met Gly Gly Met Asp Phe
                325
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<210> 43
<211> 243
<212> PRT
<213> Mycobacterium vaccae

<400> 43

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Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr
 1               5                  10                  15
Trp Thr Ala Glu Asn Ser Val Gly Ile Gly Gly Ala Tyr Leu Cys Ile
            20                  25                  30
Tyr Gly Met Glu Gly Pro Gly Gly Tyr Gln Phe Val Gly Arg Thr Thr
        35                  40                  45
Gln Val Trp Ser Arg Tyr Arg His Thr Ala Pro Phe Glu Pro Gly Ser
    50                  55                  60
Pro Trp Leu Leu Arg Phe Phe Asp Arg Ile Ser Trp Tyr Pro Val Ser
65                  70                  75                  80
Ala Glu Glu Leu Leu Glu Leu Arg Ala Asp Met Ala Ala Gly Arg Gly
                85                  90                  95
Ser Val Asp Ile Thr Asp Gly Val Phe Ser Leu Ala Glu His Glu Arg
            100                 105                 110
Phe Leu Ala Asp Asn Ala Asp Asp Ile Ala Ala Phe Arg Ser Arg Gln
        115                 120                 125
Ala Ala Ala Phe Ser Ala Glu Arg Thr Ala Trp Ala Ala Ala Gly Glu
    130                 135                 140
Phe Asp Arg Ala Glu Lys Ala Ala Ser Lys Ala Thr Asp Ala Asp Thr
145                 150                 155                 160
Gly Asp Leu Val Leu Tyr Asp Gly Asp Glu Arg Val Asp Ala Pro Phe
                165                 170                 175
Ala Ser Ser Val Trp Lys Val Asp Val Ala Val Gly Asp Arg Val Val
```

```
            180                 185                 190
Ala Gly Gln Pro Leu Leu Ala Leu Glu Ala Met Lys Met Glu Thr Val
        195                 200                 205
Leu Arg Ala Pro Ala Asp Gly Val Val Thr Gln Ile Leu Val Ser Ala
    210                 215                 220
Gly His Leu Val Asp Pro Gly Thr Pro Leu Val Val Val Gly Thr Gly
225                 230                 235                 240
Val Arg Ala
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<210> 44
<211> 370
<212> PRT
<213> Mycobacterium vaccae

<400> 44

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Met Val Arg Ala Ala Leu Arg Tyr Gly Phe Gly Thr Ala Ser Leu Leu
 1               5                  10                  15
Ala Gly Gly Phe Val Leu Arg Ala Leu Gln Gly Thr Pro Ala Ala Leu
            20                  25                  30
Gly Ala Thr Pro Gly Glu Val Ala Pro Val Ala Arg Arg Ser Pro Asn
        35                  40                  45
Tyr Arg Asp Gly Lys Phe Val Asn Leu Glu Pro Pro Ser Gly Ile Thr
    50                  55                  60
Met Asp Arg Asp Leu Gln Arg Met Leu Leu Arg Asp Leu Ala Asn Ala
65                  70                  75                  80
Ala Ser Gln Gly Lys Pro Pro Gly Pro Ile Pro Leu Ala Glu Pro Pro
                85                  90                  95
Lys Gly Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His
            100                 105                 110
Ser Ser Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro
        115                 120                 125
Val Trp Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg
    130                 135                 140
Met His Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala
145                 150                 155                 160
Val Val Ile Ser His Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile
                165                 170                 175
Val Ala Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly
            180                 185                 190
Ile Gly Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val
        195                 200                 205
Glu Leu Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val
    210                 215                 220
Cys Thr Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser
225                 230                 235                 240
Thr Leu Trp Ala Ser Trp Val Val Thr Gly Ser Ser His Lys Ala Phe
                245                 250                 255
Phe Gly Gly Asp Thr Gly Tyr Thr Lys Ser Phe Ala Glu Ile Gly Asp
            260                 265                 270
Glu Tyr Gly Pro Phe Asp Leu Thr Leu Leu Pro Ile Gly Ala Tyr His
        275                 280                 285
Pro Ala Phe Ala Asp Ile His Met Asn Pro Glu Glu Ala Val Arg Ala
    290                 295                 300
His Leu Asp Leu Thr Glu Val Asp Asn Ser Leu Met Val Pro Ile His
305                 310                 315                 320
Trp Ala Thr Phe Arg Leu Ala Pro His Pro Trp Ser Glu Pro Ala Glu
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325 330 335
Arg Leu Leu Thr Ala Ala Asp Ala Glu Arg Val Arg Leu Thr Val Pro
340 345 350
Ile Pro Gly Gln Arg Val Asp Pro Glu Ser Thr Phe Asp Pro Trp Trp
355 360 365
Arg Phe
370

<210> 45
<211> 336
<212> PRT
<213> Mycobacterium vaccae

<400> 45
Met Lys Ala Asn His Ser Gly Cys Tyr Lys Ser Ala Gly Pro Ile Trp
1 5 10 15
Ser His Pro Ser Pro Leu Cys Ser Pro Ala Leu Ala Pro Ser His Ala
20 25 30
Gly Leu Asp Asn Glu Leu Ser Leu Gly Val His Gly Gln Gly Pro Glu
35 40 45
His Leu Thr Ile Gln Gln Trp Asp Thr Phe Leu Asn Gly Val Phe Pro
50 55 60
Leu Asp Arg Asn Arg Leu Thr Arg Glu Trp Phe His Ser Gly Lys Ala
65 70 75 80
Thr Tyr Val Val Ala Gly Glu Gly Ala Asp Glu Phe Glu Gly Thr Leu
85 90 95
Glu Leu Gly Tyr His Val Gly Phe Pro Trp Ser Leu Gly Val Gly Ile
100 105 110
Asn Phe Ser Tyr Thr Thr Pro Asn Ile Thr Tyr Asp Gly Tyr Gly Leu
115 120 125
Asn Phe Ala Asp Pro Leu Leu Gly Phe Gly Asp Ser Ile Val Thr Pro
130 135 140
Pro Leu Phe Pro Gly Val Ser Ile Thr Ala Asp Leu Gly Asn Gly Pro
145 150 155 160
Gly Ile Gln Glu Val Ala Thr Phe Ser Val Asp Val Ala Gly Pro Gly
165 170 175
Gly Ser Val Val Val Ser Asn Ala His Gly Thr Val Thr Gly Ala Ala
180 185 190
Gly Gly Val Leu Leu Arg Pro Phe Ala Arg Leu Ile Ser Ser Thr Gly
195 200 205
Asp Ser Val Thr Thr Tyr Gly Ala Pro Leu Lys His Glu Leu Thr Thr
210 215 220
Ser Arg Trp Arg Pro Pro Gly Val Asn Arg Gly Pro Leu His Ala Gly
225 230 235 240
Arg Glu Ala Pro Glu Val Arg Ser Lys Trp Pro Thr Ala Ala Asn Ala
245 250 255
Cys Ala Arg Asp Ser Ser Ser Leu Thr Gln Gly Leu Val Val Val Glu
260 265 270
Cys His Pro Val Thr Pro Pro His Arg Pro Arg Arg Asp Gly Arg Gly
275 280 285
Ser Gly Val Trp Ala Pro Ala Leu Gly Thr Tyr Gly Gly Asp Arg Arg
290 295 300
Arg Asp Val Thr Ser Val Ala Val Phe Ala Gly Asn Pro Asp Gly Pro
305 310 315 320
Ala Glu Ser Pro His Pro Ser Ser Glu Pro Gly Gly Ser Lys Glu Phe
325 330 335

<210> 46
<211> 297
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> VARIANT
<222> (1)...(297)
<223> Xaa = Any Amino Acid

<400> 46
Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala
1 5 10 15
Gly Arg Pro Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala
20 25 30
Thr His Ile Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu
35 40 45
Leu Ala Gly Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His
50 55 60
Arg Leu Thr Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp
65 70 75 80
Val Cys Glu Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg
85 90 95
Thr Asp Gly Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu
100 105 110
Lys Ser Ile Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser
115 120 125
Thr Tyr Leu Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg
130 135 140
Xaa Asn Gly Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser
145 150 155 160
Ala Met Arg Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln
165 170 175
Gln Glu Ile Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu
180 185 190
Arg Leu Gln Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val
195 200 205
Asp Gly Arg Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile
210 215 220
Pro Ala Arg Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu
225 230 235 240
Thr Arg Glu Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr
245 250 255
Val Leu Glu Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys
260 265 270
Pro Ile Leu Leu His Val Ile Gly Ala Val Ala Asp Arg Arg Ala His
275 280 285
Glu Leu Arg Leu Met Asp Ser Gln Asp
290 295

<210> 47
<211> 670
<212> PRT
<213> Mycobacterium vaccae

<400> 47

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Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser Val Phe Asp Arg
 1               5                  10                  15
Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu Glu Asn Gln Phe
            20                  25                  30
Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg Gly Ser Thr Ala
        35                  40                  45
Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg Gln Leu Gly Asp
    50                  55                  60
Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg Arg Tyr Tyr Asp
65                  70                  75                  80
Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly Asn Arg Val Asp
                85                  90                  95
Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg Tyr Leu Gln Ala
            100                 105                 110
Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala Ile Ala Phe Asp
        115                 120                 125
Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn Ala Arg Phe Asn
    130                 135                 140
Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe Glu Asp Leu Met
145                 150                 155                 160
Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala Tyr Lys Gly Pro
                165                 170                 175
Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg Asn Arg Glu Leu
            180                 185                 190
Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser Ile Asp Tyr Val
        195                 200                 205
Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu Glu Pro Thr Ala
    210                 215                 220
Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val Asp Gly Val Met
225                 230                 235                 240
Ala Val Gln Phe Pro Ile Ala Arg Ile Asn Glu Leu Met Thr Ala Arg
                245                 250                 255
Gly Gln Trp Arg Asp Thr Gly Met Gly Asp Thr Gly Glu Thr Ile Leu
            260                 265                 270
Val Gly Pro Asp Asn Leu Met Arg Ser Asp Ser Arg Leu Phe Arg Glu
        275                 280                 285
Asn Arg Glu Lys Phe Leu Ala Asp Val Val Glu Gly Gly Thr Pro Pro
    290                 295                 300
Glu Val Ala Asp Glu Ser Val Asp Arg Arg Gly Thr Thr Leu Val Gln
305                 310                 315                 320
Pro Val Thr Thr Arg Ser Val Glu Glu Ala Gln Arg Gly Asn Thr Gly
                325                 330                 335
Thr Thr Ile Glu Asp Asp Tyr Leu Gly His Glu Ala Leu Gln Ala Tyr
            340                 345                 350
Ser Pro Val Asp Leu Pro Gly Leu His Trp Val Ile Val Ala Lys Ile
        355                 360                 365
Asp Thr Asp Glu Ala Phe Ala Pro Val Ala Gln Phe Thr Arg Thr Leu
    370                 375                 380
Val Leu Ser Thr Val Ile Ile Ile Phe Gly Val Ser Leu Ala Ala Met
385                 390                 395                 400
Leu Leu Ala Arg Leu Phe Val Arg Pro Ile Arg Arg Leu Gln Ala Gly
                405                 410                 415
Ala Gln Gln Ile Ser Gly Gly Asp Tyr Arg Leu Ala Leu Pro Val Leu
            420                 425                 430
Ser Arg Asp Glu Phe Gly Asp Leu Thr Thr Ala Phe Asn Asp Met Ser
        435                 440                 445
Arg Asn Leu Ser Ile Lys Asp Glu Leu Leu Gly Glu Glu Arg Ala Glu
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    450                 455                 460
Asn Gln Arg Leu Met Leu Ser Leu Met Pro Glu Pro Val Met Gln Arg
465                 470                 475                 480
Tyr Leu Asp Gly Glu Glu Thr Ile Ala Gln Asp His Lys Asn Val Thr
                485                 490                 495
Val Ile Phe Ala Asp Met Met Gly Leu Asp Glu Leu Ser Arg Met Leu
            500                 505                 510
Thr Ser Glu Glu Leu Met Val Val Val Asn Asp Leu Thr Arg Gln Phe
        515                 520                 525
Asp Ala Ala Ala Glu Ser Leu Gly Val Asp His Val Arg Thr Leu His
    530                 535                 540
Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly Val Pro Arg Leu Asp Asn
545                 550                 555                 560
Val Arg Arg Thr Val Asn Phe Ala Ile Glu Met Asp Arg Ile Ile Asp
                565                 570                 575
Arg His Ala Ala Glu Ser Gly His Asp Leu Arg Leu Arg Ala Gly Ile
            580                 585                 590
Asp Thr Gly Ser Ala Ala Ser Gly Leu Val Gly Arg Ser Thr Leu Ala
        595                 600                 605
Tyr Asp Met Trp Gly Ser Ala Val Asp Val Ala Asn Gln Val Gln Arg
    610                 615                 620
Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr Ser Arg Val His Glu Val
625                 630                 635                 640
Met Gln Glu Thr Leu Asp Phe Val Ala Ala Gly Glu Val Val Gly Glu
                645                 650                 655
Arg Gly Val Glu Thr Val Trp Arg Leu Gln Gly His Arg Arg
            660                 665                 670
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<210> 48
<211> 173
<212> PRT
<213> Mycobacterium vaccae

<400> 48

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Thr Tyr Glu Phe Glu Asn Lys Val Thr Gly Gly Arg Ile Pro Arg Glu
 1               5                  10                  15
Tyr Ile Pro Ser Val Asp Ala Gly Ala Gln Asp Ala Met Gln Tyr Gly
            20                  25                  30
Val Leu Ala Gly Tyr Pro Leu Val Asn Val Lys Leu Thr Leu Leu Asp
        35                  40                  45
Gly Ala Tyr His Glu Val Asp Ser Ser Glu Met Ala Phe Lys Val Ala
    50                  55                  60
Gly Ser Gln Val Met Lys Lys Ala Ala Ala Gln Ala Gln Pro Val Ile
65                  70                  75                  80
Leu Glu Pro Val Met Ala Val Glu Val Thr Thr Pro Glu Asp Tyr Met
                85                  90                  95
Gly Glu Val Ile Gly Asp Leu Asn Ser Arg Arg Gly Gln Ile Gln Ala
            100                 105                 110
Met Glu Glu Arg Ser Gly Ala Arg Val Val Lys Ala Gln Val Pro Leu
        115                 120                 125
Ser Glu Met Phe Gly Tyr Val Gly Asp Leu Arg Ser Lys Thr Gln Gly
    130                 135                 140
Arg Ala Asn Tyr Ser Met Val Phe Asp Ser Tyr Ala Glu Val Pro Ala
145                 150                 155                 160
Asn Val Ser Lys Glu Ile Ile Ala Lys Ala Thr Gly Gln
                165                 170
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<210> 49
<211> 187
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> VARIANT
<222> (1)...(187)
<223> Xaa = Any Amino Acid

<400> 49
Val Ile Asp Glu Thr Leu Phe His Ala Glu Glu Lys Met Glu Lys Ala
1 5 10 15
Val Ser Val Ala Pro Asp Asp Leu Ala Ser Ile Arg Thr Gly Arg Ala
20 25 30
Asn Pro Gly Met Phe Asn Arg Ile Asn Ile Asp Tyr Tyr Gly Ala Ser
35 40 45
Thr Pro Ile Thr Gln Leu Ser Ser Ile Asn Val Pro Glu Ala Arg Met
50 55 60
Val Val Ile Lys Pro Tyr Glu Ala Ser Gln Leu Arg Leu Ile Glu Asp
65 70 75 80
Ala Ile Arg Asn Ser Asp Leu Gly Val Asn Pro Thr Asn Asp Gly Asn
85 90 95
Ile Ile Arg Val Ser Ile Pro Gln Leu Thr Glu Glu Arg Arg Arg Asp
100 105 110
Leu Val Lys Gln Ala Lys Ala Lys Gly Glu Asp Ala Lys Val Ser Val
115 120 125
Arg Asn Ile Arg Arg Lys Ala Met Glu Glu Leu Ser Arg Ile Lys Lys
130 135 140
Asp Gly Asp Ala Gly Glu Asp Glu Val Thr Arg Ala Glu Lys Asp Leu
145 150 155 160
Asp Lys Ser Thr His Gln Tyr Thr Asn Gln Ile Asp Glu Leu Val Lys
165 170 175
His Lys Glu Gly Glu Leu Leu Glu Val Xaa Pro
180 185

<210> 50
<211> 331
<212> PRT
<213> Mycobacterium vaccae

<400> 50
Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Gly Gly Ile Gly
1 5 10 15
Ala Cys Ala Ala Gly Ile Ala Gly Val Leu Ser Ile Ala Val Thr Thr
20 25 30
Ala Ser Ala Gln Pro Gly Leu Pro Gln Pro Pro Leu Pro Ala Pro Ala
35 40 45
Thr Val Thr Gln Thr Val Thr Val Ala Pro Asn Ala Ala Pro Gln Leu
50 55 60
Ile Pro Arg Pro Gly Val Thr Pro Ala Thr Gly Gly Ala Ala Ala Val
65 70 75 80
Pro Ala Gly Val Ser Ala Pro Ala Val Ala Pro Ala Pro Ala Leu Pro
85 90 95
Ala Arg Pro Val Ser Thr Ile Ala Pro Ala Thr Ser Gly Thr Leu Ser
100 105 110

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Glu Phe Phe Ala Ala Lys Gly Val Thr Met Glu Pro Gln Ser Ser Arg
        115                 120                 125
Asp Phe Arg Ala Leu Asn Ile Val Leu Pro Lys Pro Arg Gly Trp Glu
    130                 135                 140
His Ile Pro Asp Pro Asn Val Pro Asp Ala Phe Ala Val Leu Ala Asp
145                 150                 155                 160
Arg Val Gly Gly Asn Gly Leu Tyr Ser Ser Asn Ala Gln Val Val Val
                165                 170                 175
Tyr Lys Leu Val Gly Glu Phe Asp Pro Lys Glu Ala Ile Ser His Gly
            180                 185                 190
Phe Val Asp Ser Gln Lys Leu Pro Ala Trp Arg Ser Thr Asp Ala Ser
        195                 200                 205
Leu Ala Asp Phe Gly Gly Met Pro Ser Ser Leu Ile Glu Gly Thr Tyr
    210                 215                 220
Arg Glu Asn Asn Met Lys Leu Asn Thr Ser Arg Arg His Val Ile Ala
225                 230                 235                 240
Thr Ala Gly Pro Asp His Tyr Leu Val Ser Leu Ser Val Thr Thr Ser
                245                 250                 255
Val Glu Gln Ala Val Ala Glu Ala Ala Glu Ala Thr Asp Ala Ile Val
            260                 265                 270
Asn Gly Phe Lys Val Ser Val Pro Gly Pro Gly Pro Ala Ala Pro Pro
        275                 280                 285
Pro Ala Pro Gly Ala Pro Gly Val Pro Pro Ala Pro Gly Ala Pro Ala
    290                 295                 300
Leu Pro Leu Ala Val Ala Pro Pro Pro Ala Pro Ala Val Pro Ala Val
305                 310                 315                 320
Ala Pro Ala Pro Gln Leu Leu Gly Leu Gln Gly
                325                 330
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<210> 51
<211> 340
<212> PRT
<213> Mycobacterium vaccae

<400> 51

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Val Thr Ile Arg Val Gly Val Asn Gly Phe Gly Arg Ile Gly Arg Asn
 1               5                  10                  15
Phe Phe Arg Ala Leu Asp Ala Gln Lys Ala Glu Gly Lys Asn Lys Asp
            20                  25                  30
Ile Glu Ile Val Ala Val Asn Asp Leu Thr Asp Asn Ala Thr Leu Ala
        35                  40                  45
His Leu Leu Lys Phe Asp Ser Ile Leu Gly Arg Leu Pro Tyr Asp Val
    50                  55                  60
Ser Leu Glu Gly Glu Asp Thr Ile Val Val Gly Ser Thr Lys Ile Lys
65                  70                  75                  80
Ala Leu Glu Val Lys Glu Gly Pro Ala Ala Leu Pro Trp Gly Asp Leu
                85                  90                  95
Gly Val Asp Val Val Val Glu Ser Thr Gly Ile Phe Thr Lys Arg Asp
            100                 105                 110
Lys Ala Gln Gly His Leu Asp Ala Gly Ala Lys Lys Val Ile Ile Ser
        115                 120                 125
Ala Pro Ala Thr Asp Glu Asp Ile Thr Ile Val Leu Gly Val Asn Asp
    130                 135                 140
Asp Lys Tyr Asp Gly Ser Gln Asn Ile Ile Ser Asn Ala Ser Cys Thr
145                 150                 155                 160
Thr Asn Cys Leu Gly Pro Leu Ala Lys Val Ile Asn Asp Glu Phe Gly
                165                 170                 175
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Ile Val Lys Gly Leu Met Thr Thr Ile His Ala Tyr Thr Gln Val Gln
            180                 185                 190
Asn Leu Gln Asp Gly Pro His Lys Asp Leu Arg Arg Ala Arg Ala Ala
        195                 200                 205
Ala Leu Asn Ile Val Pro Thr Ser Thr Gly Ala Ala Lys Ala Ile Gly
    210                 215                 220
Leu Val Leu Pro Glu Leu Lys Gly Lys Leu Asp Gly Tyr Ala Leu Arg
225                 230                 235                 240
Val Pro Ile Pro Thr Gly Ser Val Thr Asp Leu Thr Ala Glu Leu Gly
                245                 250                 255
Lys Ser Ala Thr Val Asp Glu Ile Asn Ala Ala Met Lys Ala Ala Ala
            260                 265                 270
Glu Gly Pro Leu Lys Gly Ile Leu Lys Tyr Tyr Asp Ala Pro Ile Val
        275                 280                 285
Ser Ser Asp Ile Val Thr Asp Pro His Ser Ser Ile Phe Asp Ser Gly
    290                 295                 300
Leu Thr Lys Val Ile Asp Asn Gln Ala Lys Val Val Ser Trp Tyr Asp
305                 310                 315                 320
Asn Glu Trp Gly Tyr Ser Asn Arg Leu Val Asp Leu Val Ala Leu Val
                325                 330                 335
Gly Lys Ser Leu
            340
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<210> 52
<211> 223
<212> PRT
<213> Mycobacterium vaccae

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<400> 52
Met Asn Lys Ala Glu Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser
 1                5                  10                  15
Asp Arg Arg Gln Ala Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile
            20                  25                  30
Val Arg Ala Val His Lys Gly Glu Ser Val Thr Ile Thr Gly Phe Gly
        35                  40                  45
Val Phe Glu Gln Arg Arg Arg Ala Ala Arg Val Ala Arg Asn Pro Arg
    50                  55                  60
Thr Gly Glu Thr Val Lys Val Lys Pro Thr Ser Val Pro Ala Phe Arg
65                  70                  75                  80
Pro Gly Ala Gln Phe Lys Ala Val Val Ser Gly Ala Gln Lys Leu Pro
                85                  90                  95
Ala Glu Gly Pro Ala Val Lys Arg Gly Val Thr Ala Thr Ser Thr Ala
            100                 105                 110
Arg Lys Ala Ala Lys Lys Ala Pro Ala Lys Lys Ala Ala Ala Lys Lys
        115                 120                 125
Ala Ala Pro Ala Lys Lys Ala Pro Ala Lys Lys Ala Ala Thr Lys Ala
    130                 135                 140
Ala Pro Ala Lys Lys Ala Thr Ala Ala Lys Lys Ala Ala Pro Ala Lys
145                 150                 155                 160
Lys Ala Thr Ala Ala Lys Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala
                165                 170                 175
Lys Lys Ala Ala Thr Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala Lys
            180                 185                 190
Lys Ala Ala Thr Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala Ala Lys
        195                 200                 205
Lys Ala Pro Ala Lys Lys Ala Pro Ala Lys Arg Gly Gly Arg Lys
    210                 215                 220
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ02/00135

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 39/04, A61K 31/715, A61K 31/738, A61K 38/16; A61P 17/06, A61P 37/02

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)
Derwent WPAT and Medline keywords: notch, signalling, toll()like()receptor, peptidoglycan, vaccae, smegmatis, tuberculosis, tolerance and immune

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Takeuchi Osamu and Akira Shizuo "Toll-like receptors; their physiological role and signal transduction system" **International Immunopharmacology, Vol. 1, (2001), pg. 625-635.** See whole document.	13
X	Wang Qiuling et al. "Micrococci and Peptidoglycan Activate TLR2→MyD88→IRAK→TRAF→NIK→IKK→NF-κB Signal Transduction Pathway That Induces Transcription of Interleukin-8" **Infection and Immunity, Vol. 69, No. 4, (April 2001), pg. 2270-2276.** See whole document.	13

[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
17 October 2002	23 OCT 2002

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ02/00135

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/74715 A (Genesis Research & Development Corporation Limited) 14 December 2000 See whole document.	4 and 5
X	WO 99/32634 A (Genesis Research & Development Corporation Limited) 1 July 1999 See whole document.	3,4 and 5
X	WO 92/08488 A (University College London) 29 May 1992 See whole document.	3
X	US 6,328,978 B (Genesis Research & Development Corporation Limited) 11 December 2001 See whole document.	4 and 5
A	Koch Ute et al. "Subversion of the T/B Lineage Decision in the thymus by Lunatic Fringe-Mediated Inhibition of Notch-1" **Immunity, Vol. 15, (August, 2001), pg. 225-236.** See whole document.	1-14
P,X	Carl Virginia S et al. "Toll-like Receptor 2 and 4 (TLR2 and TLR4) Agonists Differentially Regulate Secretory Interleukin-1 Receptor Antagonist Gene Expression in Macrophages" **The Journal of Biological Chemistry, Vol. 277, No. 20, (17 May 2002), pg. 17448-17456.** See whole document.	13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ02/00135

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	200074715	AU	200052579	BR	200011239	EP	1181051
		US	6350457				
WO	9932634	AU	18936/99	BR	9814432	CA	2315539
		EP	1044273	HU	200100352	NO	20003261
		PL	341697	US	5968524	US	6328978
		US	5985287	US	6160093	US	6406704
		AU	40365/97	BR	9711457	CN	1235555
		EP	939646	NZ	334358	US	6284255
		WO	9808542	US	6410720	US	6001361
		ZA	9801148				
WO	9208488	AU	88741/91	CA	2095855	EP	556248
		US	5599545				

END OF ANNEX