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<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; vertical-align: top; padding: 5px;"> (21) International Application Number: PCT/AU99/00929 (22) International Filing Date: 27 October 1999 (27.10.99) (30) Priority Data: PP 6758 27 October 1998 (27.10.98) AU (71) Applicant (for all designated States except US): THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Parade, Parkville, Victoria 3052 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): SCHOFIELD, Louis [AU/AU]; 185 Panorama Drive, Gisborne, Victoria 3437 (AU). HANSEN, Diana [AR/AU]; 5 Ladner Court, Chadstone, Victoria 3148 (AU). (74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, Victoria 3000 (AU). </td> <td style="width: 50%; vertical-align: top; padding: 5px;"> (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. </td> </tr> </table>			(21) International Application Number: PCT/AU99/00929 (22) International Filing Date: 27 October 1999 (27.10.99) (30) Priority Data: PP 6758 27 October 1998 (27.10.98) AU (71) Applicant (for all designated States except US): THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Parade, Parkville, Victoria 3052 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): SCHOFIELD, Louis [AU/AU]; 185 Panorama Drive, Gisborne, Victoria 3437 (AU). HANSEN, Diana [AR/AU]; 5 Ladner Court, Chadstone, Victoria 3148 (AU). (74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, Victoria 3000 (AU).	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report.
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(54) Title: A METHOD OF ACTIVATING T CELLS AND AGENTS USEFUL FOR SAME				
(57) Abstract The present invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (referred to herein as "GPI") molecules and derivatives or equivalents thereof. Even more particularly the method of the present invention contemplates a method of activating T cells, using GPI molecules, via a CD1-restricted pathway. The method of the present invention is useful, <i>inter alia</i>, in a range of therapeutic and/or prophylactic applications including, but not limited to applications which require skewing of the TH1/TH2 response or which require the induction of antibody production.				
Parasite protein anchors (PfGPI, mfVSG GPI) Leishmanial free GPIs Synthetic Thy-1 anchor				

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A METHOD OF ACTIVATING T CELLS AND AGENTS USEFUL FOR SAME

FIELD OF THE INVENTION

5 The present invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (referred to herein as "GPI") molecules and derivatives or equivalents thereof. Even more particularly the method of the present invention contemplates a method of activating T cells, using GPI molecules, via a CD1-restricted pathway. The method of the present
10 invention is useful, inter alia, in a range of therapeutic and/or prophylactic applications including, but not limited to applications which require skewing of the TH1/TH2 response or which require the induction of antibody production.

BACKGROUND OF THE INVENTION

15

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

Antibody responses to protein are understood to be MHC restricted. That is, the
20 production of antibodies directed to a given T dependent antigen requires the production of cytokines by stimulated TH2 cells. Said TH2 cells are stimulated following their binding to a MHC II/peptide complex comprising a peptide derived from the processing of said antigen. Since MHC molecules are polymorphic, there exist genetically determined high and low responders to peptide vaccines.

25

GPI anchor surface proteins occur frequently among medically important parasitic and fungal taxa such as *Plasmodium*, *Trypanosoma*, *Leishmania*, *Toxoplasma* and *Candida*.

GPIs are ubiquitous among eukaryotes, described from *T. brucei*, *T. cruzi*, *Plasmodium*,
30 *Leishmania*, and *Toxoplasma*, as well as yeast, insect, fish and numerous mammalian sources (for recent reviews see (1, 2)). GPIs consist of a conserved core glycan (Man α 1-

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2Man α 1-6Man α 1-4GlcNH₂ linked to the 6-position of the *myo*-inositol ring of PI. GPIs are built up on the cytoplasmic face of the endoplasmic reticulum by the sequential addition of sugar residues to PI by the action of glycosyltransferases. The maturing GPI is then translocated across the membrane to the luminal side of the ER, whence it may be exported
5 to the cell surface, free or in covalent association with proteins. The terasaccharide core glycan may be further substituted with sugars, phosphates and ethanolamine groups in a species and tissue-specific manner. GPI fatty acid moieties can be either diacylglycerols, alkylacylglycerols, monoalkylglycerols or ceramides, with additional palmitoylations or myristoylations to the inositol ring. The overall picture is of a closely related family of
10 glycolipids sharing certain core features but with a high level of variation in fatty acid composition and side-chain modifications to the conserved core glycan.

In work leading up to the present invention, the inventors have shown that the antibody response to several parasitic proteins is regulated predominantly through CD1-restricted
15 recognition of the covalently associated GPI moiety by IL-4 producing CD4⁺ T cells with limited TCR repertoire diversity. In contrast, there is little evidence for MHC II restricted T cell responses to these antigens. GPI moieties, therefore, act as universal T cell sites through presentation by the non-polymorphic CD1 restriction element.

20 SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the
25 exclusion of any other integer or group of integers.

One aspect of the present invention contemplates a method of activating T cells, said method comprising administering a T cell activating effective amount of a molecule or a complex comprising said molecule which molecule or molecule complex is capable of
30 interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

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Another aspect of the present invention contemplates a method of activating T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form
 5 an association with CD1 which association activates helper T cells.

Yet another aspect of the present invention contemplates a method of activating helper T cells said method comprising administering a T cell activating effective amount of *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said
 10 *Plasmodium* GPI or derivative or equivalent thereof which *Plasmodium* GPI or *Plasmodium* GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

In still another aspect, said GPI comprises a structure selected from:

15

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y

20

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 25 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another aspect, said GPI comprises a structure selected from:

30

EtN-P-M α 2M α 6M α 4G-Y

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M α 2M α 6M α 4G-YEtN-P-M α 2M α 6M-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 5 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 10 is any lipid or phospholipid.

In yet still another aspect, said GPI comprises a structure selected from:

EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y15 EtN-P-[M α 2][X]M α 2M α 6M α 4G-YEtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 is any lipid or phospholipid.

25

In a further aspect, said GPI comprises a structure selected from:

M α 2[M α 2][G]M α 2M α 6M α 4G-YM α 2[M α 2][X]M α 2M α 6M α 4G-Y30 M α 2[M α 2][EtN-P]M α 6M α 4G-Y

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or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 5 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

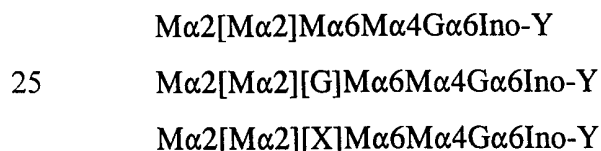
In another further aspect, said GPI comprises a structure selected from:

10



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 15 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y
 20 is any lipid or phospholipid.

In still another further aspect, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 30 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may

- 6 -

be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

5 In still yet another further aspect, said GPI comprises a structure selected from:



10

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
15 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In yet still another further aspect, said GPI comprises a structure selected from:

20



25 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
30 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

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In still yet another further aspect, said GPI comprises a structure selected from:

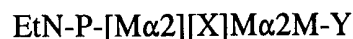
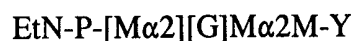


5

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 10 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another aspect, said GPI comprises a structure selected from:

15



20 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 25 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

Another aspect of the present invention contemplates a method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or
 30 derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an

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immune cell to form an association with CD1 which association activates CD4⁺ NK1.1⁺ T cells.

Yet another aspect of the present invention contemplates a method of activating CD4⁺,
5 NK1.1⁺ T cells said method comprising administering a T cell activating effective amount
of a *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said
Plasmodium GPI or derivative or equivalent thereof which *Plasmodium* GPI or
Plasmodium GPI complex is capable of interacting with CD1 on an immune cell to form
an association with CD1 which association activates CD4⁺ NK1.1⁺ T cells.

10

Still another aspect of the present invention contemplates a method of activating T helper
cells said method comprising administering a T cell activating effective amount of a GPI
or derivative or equivalent thereof or a complex comprising said GPI or derivative or
equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an
15 immune cell to form an association with CD1 which association activates T helper cells
wherein said activated T cells provide B cell help.

Still yet another aspect of the present invention contemplates a method of activating CD4⁺,
NK1.1⁺ T cells said method comprising administering a T cell activating effective amount
20 of a *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said
Plasmodium GPI or derivative or equivalent thereof which *Plasmodium* GPI or
Plasmodium GPI complex is capable of interacting with CD1 on an immune cell to form
an association with CD1 which association activates CD4⁺, NK1.1⁺ T cells wherein said
activated T cells provide B cell help.

25

Yet still another aspect of the present invention contemplates a method of activating T
helper cells said method comprising administering a T cell activating effective amount of
a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative
or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an
30 immune cell to form an association with CD1 which association activates T helper cells
wherein said activated T cells induce or otherwise upregulate a TH1-type response.

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A further aspect of the present invention contemplates a method of activating T helper cells said method comprising administering a T cell activating effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or
5 equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates T helper cells wherein said activated T cells induce or otherwise upregulate a TH2-type response.

Another further aspect of the present invention provides a method of inducing, in a
10 mammal, an immune response directed to a GPI said method comprising administering to said mammal a T cell activating effective amount of GPI or derivative or equivalent thereof which GPI is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

15 Still another further aspect of the present invention provides a method of inducing, in a mammal, an immune response directed to an antigen, said method comprising administering to said mammal a T cell activating effective amount of GPI or derivative or equivalent thereof complexed to said antigen, which GPI-antigen complex is capable of interacting with CD1 on an immune cell to form an association with CD1, which
20 association activates helper T cells.

Still yet another further aspect of the present invention contemplates a method of treating a mammal said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or
25 equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

Yet still another further aspect of the present invention contemplates a method of treating a mammal said method comprising administering a mammalian helper T cell activation
30 effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting

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with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

Another aspect of the present invention contemplates the use of GPI or derivative or
5 equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof
which GPI or GPI complex is capable of interacting with CD1 on an immune cell, in the
manufacture of a medicament for the activation of helper T cells in a mammal.

Yet another aspect of the present invention provides a method for the treatment and/or
10 prophylaxis of a mammalian disease condition characterised by a micro-organism infection,
said method comprising administering to said mammal an effective amount of GPI or
derivative or equivalent thereof or a complex comprising said GPI or derivative or
equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an
immune cell to form an association with CD1 which association activates helper T cells.

15

Still another aspect of the present invention provides a method for the treatment and/or
prophylaxis of a mammalian disease condition characterised by the insufficiency or absence
of an appropriate TH1 response said method comprising administering to said mammal an
effective amount of GPI or derivative or equivalent thereof or a complex comprising said
20 GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting
with CD1 on an immune cell to form an association with CD1 which association induces
or otherwise upregulates a TH1 response.

Still yet another aspect of the present invention provides a method for the treatment and/or
25 prophylaxis of a mammalian disease condition characterised by the insufficiency or absence
of an appropriate TH2 response said method comprising administering to said mammal an
effective amount of GPI or derivative or equivalent thereof or a complex comprising said
GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting
with CD1 on an immune cell to form an association with CD1 which association induces
30 or otherwise upregulates a TH2 response.

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Yet still another aspect of the present invention is directed to a composition which activates T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1
5 which association activates helper T cells.

A further aspect of the present invention relates to an immunogenic composition comprising as the active component GPI or derivative or equivalent thereof or GPI complex, as broadly described above.

10

Another further aspect of the present invention is directed to a pharmaceutical composition capable of activating T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an
15 association with CD1, which association activates helper T cells, together with one or more pharmaceutically acceptable carriers and/or diluents.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation of the survival from PbA infection in Balb/c mice wild type (○), Balb/c mice CD1d^{-/-} (●), and C57B/6 (▲) mice. The results are from a single experiment representative of 2 independent experiments.

Figure 2 is a graphical representation of the percentage of CD4⁺ cells producing IL-4 or IFN- γ during the infection with PbA. Balb/c wild type (□) and Balb/c CD1^{-/-} (■) mice were infected with 1 x 10⁶ PbA infected red blood cells. At day 7 post-infection the animals were sacrificed and the spleen cells stained for CD4⁺ and intracellular cytokine content. The percentage of CD4⁺ cells producing IL-4 or IFN- γ is indicated.

Figure 3. Diagrammatical representation of GPI structures used in this study. Purification and compositional analyses are as described. (A) COOH-terminal GPIs from *T. brucei* and *P. falciparum*. Boxed areas represent modifications found in PfGPI. The cleavage site of mfVSG by phosphatidylinositol-specific phospholipase C (PI-PLC) is indicated. (B) Free iM2 iM4 and EP-iM4 GPIs of *L. mexicana*. Nomenclature is as described, where all isomers contain one mannose in al-3 linkage, EP indicates ethanolamine phosphate, and M2, M4 indicate number of mannose residues, as shown. (C) Chemically synthesized rat brain Thy-1 GPI.

Figure 4. Response of peripheral NKT cells to purified GPIs *in vitro*. (A) As determined by forward (FSC) and side (SSC) light scatter, splenocytes from SPZ-primed class II^{-/-} donors proliferate within 48h. exposure to PfGPI (unshaded) compared with medium controls (shaded). The responding cells are NK1.1⁺, CD4⁺, and include a V α 14⁺, CD4⁺ subset. (B) Splenocytes from class II^{-/-} donors were exposed to various antigens and [³H]TdR incorporation determined after 4 days. Other cultures were exposed to PfGPI or Thy-1 GPI for 4 days, washed and cultured in 10U/ml IL-2 for 2 days, followed by replating with irradiated wild-type APCs and restimulation with either PfGPI or Thy-1 GPI for 48 hours. IL-4 levels in the supernatant were determined by capture ELISA.

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Figure 5. The proliferative and IL-4 response of NKT cells to PfGPI is MHC-independent and CD1-restricted. (A) 2×10^4 sorted NK1.1⁺, CD4⁺ cells from wild-type or class II^{-/-} donors were placed in triplicate with or without purified GPI on irradiated splenocyte APCs from wild-type (WT), class II^{-/-}, b₂M^{-/-} or CD1^{-/-} donors, or CD1.1-transfected and sham-transfected J774 macrophages. Tritiated thymidine ([³H]TdR) incorporation was determined after 3 days, or IL-4 production in the presence or absence of anti-CD1 was determined as above. (B) Splenocytes from SPZ-primed Class II^{-/-} donors were exposed to PfGPI in the presence or absence of anti-CD1 or isotype control and taken for FACS analysis after 3 days.

10

Figure 6. CD1-restricted antibody formation to neo-GPI-proteins and malaria SPZ. (A) Donor *nu/nu* mice were primed twice with *P. berghei* SPZ or twice with LPS^{FLU}. Splenocytes were cultured in the presence of 10U/ml IL-2, with and without antigen (0.1mg/ml sham-OVA^{FLU}, PfGPI-OVA^{FLU}, or 5×10^4 SPZ), anti-Class I, anti-Class II and anti-CD1, with 10^4 NKT cells from SPZ-primed Class II^{-/-} donors. Antigen-specific IgG production was quantified by ELISPOT against fluoresceinated Dog serum albumin for responses to OVA^{FLU}, and rCS for responses to SPZ. (B) Responses of CD1.1/CD1.2^{-/-} (open circles) and Balb/c wild-type mice (black circles) to SPZ, recCS and mfVSG^{FLU}. IgG titers were determined as described.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the surprising determination that the helper T cell response to several parasitic proteins is predominantly regulated through CD1-
5 restricted recognition of the GPI moiety by CD4+ T cells. This determination has facilitated the development of methodology for application, inter alia, in improving immune responses via regulation of helper T cell stimulation, for example, where the immune response is desired for the purpose of therapeutic or prophylactic vaccination.

10 Accordingly, one aspect of the present invention contemplates a method of activating T cells, said method comprising administering a T cell activating effective amount of a molecule or a complex comprising said molecule which molecule or molecule complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

15

Preferably, the present invention contemplates a method of activating T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an
20 association with CD1 which association activates helper T cells.

Reference hereinafter to "activating" helper T cells is a reference to upregulating one or more of the functions which said T cells are capable of performing upon stimulation by an antigen, such as, but not limited to, one or more of cell division, differentiation, cell
25 surface molecule expression or cytokine production.

Reference to "association" should be understood in its broadest sense to include any form of interaction between a molecule and CD1. Said molecule and CD1 may interact via, for example, a covalent bond, ionic bond, hydrogen bond, van Der Waals forces or other
30 interactive bonding mechanism.

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To the extent that it is not otherwise specified, reference to "GPI" should be read as including reference to all forms of GPI and derivatives or equivalents thereof. Reference to "derivatives" or "equivalents" should be understood to include reference to fragments, parts, portions, chemical equivalents, mutants, homologs and analogs. Chemical
5 equivalents of GPI can act as a functional analog of GPI. Equivalents may not necessarily be derived from GPI but may share certain conformational similarities. Alternatively chemical equivalents may be specifically designed to mimic certain physiochemical properties of GPI. Chemical equivalents may be chemically synthesised or may be detected following, for example, natural product screening. Equivalents also include
10 synthetic carbohydrates and peptide mimetics. Homologs of GPI contemplated herein include, but are not limited to, GPI from different species.

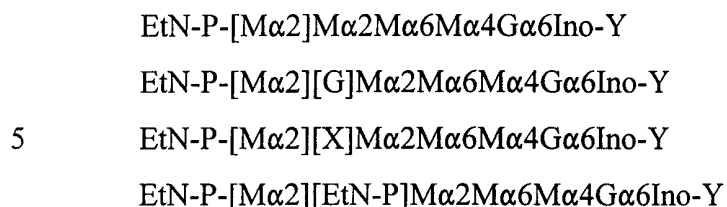
GPI molecules suitable for use in the present invention may be derived from any natural or synthetic source. This includes, for example, GPI moieties derived by genetic
15 manipulation of expression systems and by manipulations of the GPI post-translational modification of proteins via recombinant DNA techniques such as glycosylation inhibitors. Examples of GPI moieties suitable for use in the present invention include but are not limited to microorganism GPI moieties which cause disease conditions such as, the parasitic, fungal and yeast taxa *Plasmodium*, *Trypanosoma*, *Leishmania*, *Toxoplasma* and
20 *Candida*. Preferably, said GPI is a parasite GPI and even more preferably a *Plasmodium* GPI.

According to this preferred embodiment, the present invention contemplates a method of activating helper T cells said method comprising administering a T cell activating effective
25 amount of *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said *Plasmodium* GPI or derivative or equivalent thereof which *Plasmodium* GPI or *Plasmodium* GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

30 Most preferably said *Plasmodium* is *P. falciparum*.

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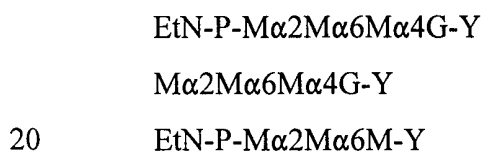
In another most preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
10 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15

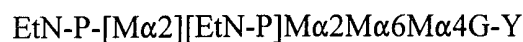
In another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
25 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

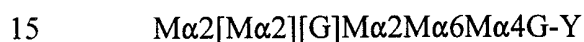
30 In still another preferred embodiment, said GPI comprises a structure selected from:

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5 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 10 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In yet another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 20 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y
 25 is any lipid or phospholipid.

In still yet another preferred embodiment, said GPI comprises a structure selected from:

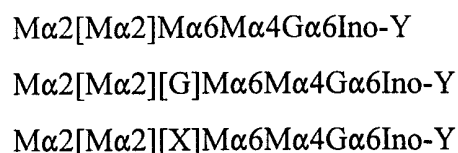


- 18 -

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

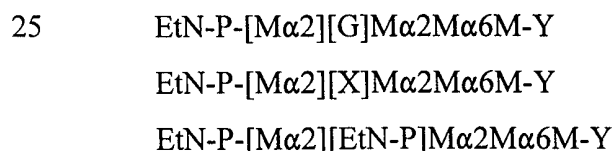
In a further embodiment, said GPI comprises a structure selected from:

10



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another further preferred embodiment, said GPI comprises a structure selected from:



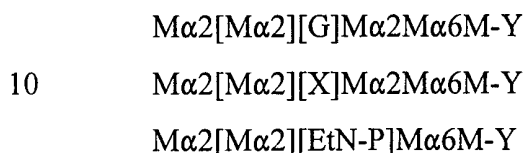
or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or

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inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

5

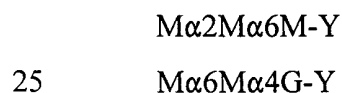
In still another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
15 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

20

In still yet another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
30 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional

- 20 -

linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:

5

EtN-P-[M α 2][G]M α 2M-Y

EtN-P-[M α 2][X]M α 2M-Y

EtN-P-[M α 2][EtN-P]M α 2M-Y

10 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
15 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

Reference to "lipid" or "phospholipid" should be understood in its broadest sense and includes, but is not limited to, diacylglycerol, alkylacylglycerol, monoalkylglycerol,
20 ceramide, sphingolipids and phospholipids such as phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine.

It should also be understood any of these preferred structures may be further modified by substituents of positive, negative or neutral charge such as phosphates, phosphoglycerol,
25 hexosamines, amino acids, thiols etc in any position and with any type of linkage. This may be particularly useful in the generation of self GPI structures which are typically more highly substituted along the length of the glycan than protozoal GPIs.

GPI "complex" is a reference to a GPI moiety coupled to any other molecule. Said
30 molecule may be any molecule to which an immune response is sought, for example, a carbohydrate or a peptide, polypeptide or protein such as, but not limited to, peptides,

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polypeptides or proteins naturally anchored to GPI moieties (for example, malarial CS protein, MSP-1, MSP-2, *Leishmanial* PSA-2 or GP63) or any peptides, polypeptides or proteins artificially coupled to a GPI moiety (for example an influenza antigen). Said molecule and said GPI moiety may be covalently linked or may be linked by ionic, hydrogen or other interactive bonding mechanisms. Coupling may be achieved by a variety of techniques including, but in no way limited to, use of a specific expression system or via chemical synthesis. Preferably, said molecule is a protein.

Reference to an "immune cell" should be understood as a reference to any cell of the immune system such as, but not limited to, myeloid cells, stromal cells or antigen presenting cells (for example macrophages).

Reference to "helper T cells" should be understood as a reference to any cell expressing a T cell receptor (expression of a "T cell receptor" is defined as the expression of one or more of an α , β , γ and/or δ T cell receptor chain in either homodimeric or heterodimeric form) which can become activated via a CD1-restricted recognition pathway instead of, or in addition to, a capacity to become activated via a MHC II restricted recognition pathway and which acts to stimulate, upregulate or otherwise modulate any aspect of the immune response via any one or more of a variety of mechanisms including, for example, cell-cell contact or production of soluble mediators. Said T cells include, but are not limited to, thymically derived T cells. Preferably said T cells express CD4 and even more preferably CD4 and NK1.1. This cell type is often referred to in the art as the "NKT cell".

According to this most preferred embodiment, the present invention contemplates a method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates CD4+ NK1.1+ T cells.

30

Without limiting the present invention to any one theory or mode of action, administration

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of GPI or a GPI complex leads to presentation of the GPI moiety, or a molecule complexed to the GPI moiety, by a non-polymorphic CD1 restriction element. CD1 is expressed on a variety of cells including, for example, macrophages. Coupling of the GPI moiety (or the molecule complexed to the GPI) to the CD1 may be by covalent bonding. Recognition
5 of the CD1-GPI unit by a subclass of CD4⁺ T cells leads to their activation. Said T cells do not recognise the GPI moiety or the molecule complexed to the GPI via the traditional MHC II restricted route of presentation, instead said T cells are activated through CD1-restricted recognition. Said T cells represent a subset of the T cell population of an individual. For example, T cells exhibiting the phenotype CD4⁺, NK1.1⁺ are able to
10 become activated via the CD1-restricted recognition pathway. The present invention should be understood to extend to methods of activating T cells by administration of GPI or GPI complex wherein said T cells are activated by one or both of CD1 or MHC II restricted recognition.

15 Even more preferably the present invention contemplates a method of activating CD4⁺, NK1.1⁺ T cells said method comprising administering a T cell activating effective amount of a *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said *Plasmodium* GPI or derivative or equivalent thereof which *Plasmodium* GPI or *Plasmodium* GPI complex is capable of interacting with CD1 on an immune cell to form
20 an association with CD1 which association activates CD4⁺ NK1.1⁺ T cells.

Most preferably said *Plasmodium* is *P. falciparum*.

Still without limiting the present invention to any one theory or mode of action, the T cells
25 of the present invention can co-operate with B cells to result in CD1-restricted antibody production, the specificity of said antibody being directed to the GPI moiety (where said GPI is administered in isolation) or to a molecule complexed to the GPI moiety (where a GPI complex is administered). For example, said complexed molecule may be a protein to which an antibody response is desired, such as an influenza antigen. Activation of said
30 B cells is supported by the production of cytokines, such as IL-4, by the CD1-restricted activated T cells. GPI-anchoring, therefore, permits antibody formation by a non-MHC

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restricted immunological process. Unlike MHC, which is a highly polymorphic molecule, CD1 is non-polymorphic thereby resulting in little observed non-responsiveness in human populations.

- 5 Accordingly, the present invention contemplates a method of activating T helper cells said method comprising administering a T cell activating effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates T helper cells
- 10 wherein said activated T cells provide B cell help.

Preferably said T helper cells are CD4⁺, NK1.1⁺ T cells.

- More preferably, said GPI molecule is a parasitic, fungal or yeast GPI moiety. Most
- 15 preferably, said GPI molecule is a parasite GPI and even more preferably a *Plasmodium* GPI.

- Accordingly, a related aspect of the present invention contemplates a method of activating CD4⁺, NK1.1⁺ T cells said method comprising administering a T cell activating effective
- 20 amount of a *Plasmodium* GPI or derivative or equivalent thereof or a complex comprising said *Plasmodium* GPI or derivative or equivalent thereof which *Plasmodium* GPI or *Plasmodium* GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates CD4⁺, NK1.1⁺ T cells wherein said activated T cells provide B cell help.

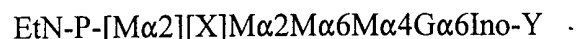
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Most preferably said *Plasmodium* is *P. falciparum*.

In another most preferred embodiment, said GPI comprises a structure selected from:

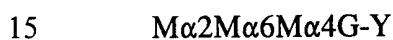
- 30 EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y

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or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 5 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 10 is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 is any lipid or phospholipid.

25

In still another preferred embodiment, said GPI comprises a structure selected from:



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- 25 -

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In yet another preferred embodiment, said GPI comprises a structure selected from:

10



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another preferred embodiment, said GPI comprises a structure selected from:

25

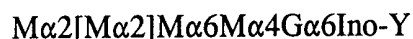


or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may

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be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

5 In a further embodiment, said GPI comprises a structure selected from:



10

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 15 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another further preferred embodiment, said GPI comprises a structure selected from:

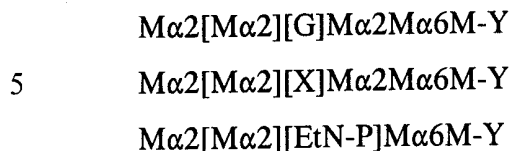
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25 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 30 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

- 27 -

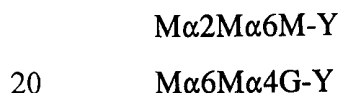
In still another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15

In still yet another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

30 In another preferred embodiment, said GPI comprises a structure selected from:

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EtN-P-[M α 2][G]M α 2M-YEtN-P-[M α 2][X]M α 2M-YEtN-P-[M α 2][EtN-P]M α 2M-Y

5 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
10 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

Reference to "B cell help" should be understood as a reference to the provision, by said activated T cells, of signals which act to stimulate, up-regulate or otherwise modulate or
15 maintain B cell and/or plasma cell viability or functional activity. Said signals may take any form including, for example, cell/cell contact or the production of soluble mediators such as cytokines.

In addition to co-operating with B cells to support antibody production, the T cells
20 activated in accordance with the method of the present invention produce a range of cytokines which can modulate aspects of the immune response other than antibody production. For example, and without limiting the present invention in any way, CD-1 restricted NKT cells produce very high levels of the cytokines IL-4 and IFN- γ suggesting that these cells are involved in the downstream regulation of TH1/TH2 differentiation.
25 Accordingly, these cells are thought to be involved in the aetiology of those diseases which show a pronounced TH1/TH2 dependence, including cerebral malaria, tuberculosis, leprosy, *leishmaniasis*, type I diabetes, autoimmune arthritis and systemic lupus erythromatosis. They are also thought to be involved in tumour rejection.

30 The up-regulation of NKT cell proliferation and differentiation therefore provides a mechanism for skewing the T helper cell response towards a TH1 or a TH2 response.

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Skewing of the CD1 restricted T helper cell response, for example, may be achieved by techniques which are known to those skilled in the art. These techniques include, for example, the route by which the GPI molecule/GPI complex is administered, co-stimulatory signals which are provided together with the GPI molecule/GPI complex
5 and/or the choice of vehicle which is used to administer the GPI molecule/GPI complex. For example, administration of the GPI molecule/GPI complex is alum or liposomes will skew the CD1 restricted T helper response towards a TH2 type response while administration of these molecules utilising ISCOM's will skew the response to either the TH1 or TH2 type response depending on the route of administration which is utilised. The
10 administration of an IL-12 or IFN- α co-stimulatory agent together with the GPI molecule/GPI complex is likely to skew the response towards a TH1 type response while the administration of an IL-4 co-stimulatory agent together with the GPI molecule/GPI complex is more likely to skew the response towards a TH2 type response.

15 The method of the present invention therefore provides not only a mechanism of activating T helper cells in a non-MHC restricted fashion in response to a GPI stimulus, but the method may also be adapted to skew the nature of the immune response which is ultimately effected. For example, up-regulation of the subject T cells can be designed to provide B cell help or to otherwise effect a TH1 or TH2 type response.

20

Accordingly, another related aspect of the present invention contemplates a method of activating T helper cells said method comprising administering a T cell activating effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with
25 CD1 on an immune cell to form an association with CD1 which association activates T helper cells wherein said activated T cells induce or otherwise upregulate a TH1-type response.

Preferably said T helper cell is a CD4⁺ NK 1.1⁺ cell. More preferably said GPI molecule
30 is a parasitic, fungal or yeast GPI moiety and even more preferably a *Plasmodium* GPI.

- 30 -

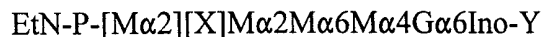
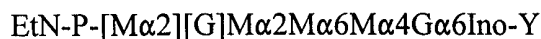
Yet another related aspect of the present invention contemplates a method of activating T helper cells said method comprising administering a T cell activating effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an
 5 immune cell to form an association with CD1 which association activates T helper cells wherein said activated T cells induce or otherwise upregulate a TH2-type response.

Preferably said T helper cell is a CD4⁺ NK1.1⁺ cell. More preferably said GPI molecule is a parasitic, fungal or yeast GPI moiety and even more preferably a *Plasmodium* GPI.

10

Reference to a "TH1-type" or "TH2-type" response should be understood as a reference to a T helper cell response which exhibits one or more of the functional characteristics of a TH1 or TH2 response, respectively, and which is directly or indirectly mediated by the CD1 restricted T cells which are activated in accordance with the method of the present
 15 invention. These characteristics include, but are not limited to the cytokine profile which is induced in the subject. For example, a TH1 response is characterised by the up-regulation of IFN- γ expression while a TH2 response is characterised by the up-regulation of IL-4 production.

20 In a most preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 30 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional

- 31 -

linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:

5

EtN-P-M α 2M α 6M α 4G-Y

M α 2M α 6M α 4G-Y

EtN-P-M α 2M α 6M-Y

10 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
15 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still another preferred embodiment, said GPI comprises a structure selected from:

20

EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y

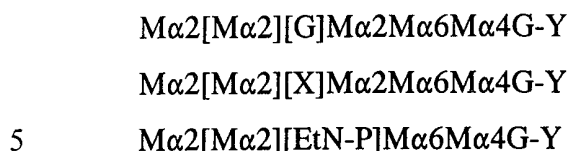
EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y

EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
25 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y
30 is any lipid or phospholipid.

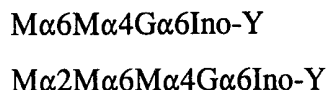
- 32 -

In yet another preferred embodiment, said GPI comprises a structure selected from:



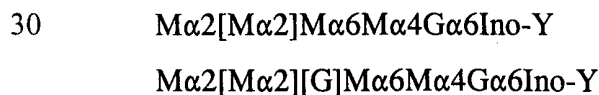
or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
10 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15 In still yet another preferred embodiment, said GPI comprises a structure selected from:



20 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
25 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In a further embodiment, said GPI comprises a structure selected from:



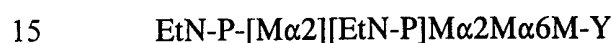
- 33 -



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 5 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

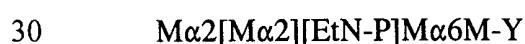
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In another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

25 In still another further preferred embodiment, said GPI comprises a structure selected from:



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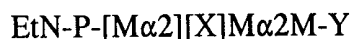
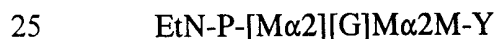
or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or

- 35 -

inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

5

A further aspect of the present invention relates to the use of the invention in relation to disease conditions. For example:

- 10 (i) As therapeutic or prophylactic immunogenic compositions directed to molecules naturally GPI-anchored such as, for example, the malarial CS protein, MSP-1, and MSP-2, *Leishmanial* PSA-2 and gp63.
- (ii) As therapeutic or prophylactic immunogenic compositions directed to GPI molecules themselves, for example in treating parasitic, fungal and yeast infections.
- 15 (iii) To enable antibody production where MHC II restricted T cell activation is undesirable, such as where said MHC II restricted T cell would engender an autoimmune reaction. For example, the M protein of Group A Streptococcus exhibits T cell epitopes which are cross-reactive with human heart tissue but
20 exhibits B cell epitopes protective against the bacterium. Said B cell epitope may be coupled to GPI to facilitate non-MHC restricted antibody formation.
- (iv) To provide a universal T cell epitope for any therapeutic or prophylactic immunogenic compositions construct.

25

In one example, the use of the present invention to regulate a TH1/TH2 response provides a mechanism to therapeutically or prophylactically treat disease conditions which show a pronounced TH1/TH2 dependence such as, but not limited to, cerebral malaria, tuberculosis, leprosy, *leishmaniasis*, type I diabetes, autoimmune arthritis, systemic lupus
30 erythromatosis. In particular, skewing towards or inducing a TH1 response in subjects suffering from neoplasia, cancer or *Leishmaniasis* is desirable. Skewing towards or

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induction of a TH2 response is desirable where a subject is suffering from SLE, type I diabetes, autoimmune arthritis and cerebral malaria, for example. The method of the present invention may also be utilised to provide B cell help, thereby leading to an antibody response which is useful, for example, in the treatment or prophylaxis of parasitic
5 infections such as malaria and *leishmaniasis*. The production of B cell help can be achieved, for example, by inducing a TH2 response.

Accordingly, in another aspect, the present invention provides a method of inducing, in a mammal, an immune response directed to a GPI said method comprising administering
10 to said mammal a T cell activating effective amount of GPI or derivative or equivalent thereof which GPI is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

Preferably, said T cell is a CD4+ T cell and even more preferably a CD4+ NK1.1+ T
15 cell. In another preferred embodiment, said activated T cell provides B cell help.

Still more preferably said GPI is *Plasmodium* GPI.

In still another aspect, the present invention provides a method of inducing, in a mammal,
20 an immune response directed to an antigen, said method comprising administering to said mammal a T cell activating effective amount of GPI or derivative or equivalent thereof complexed to said antigen, which GPI-antigen complex is capable of interacting with CD1 on an immune cell to form an association with CD1, which association activates helper T cells.

25

Preferably, said T cell is a CD4+ T cell and even more preferably a CD4+ NK1.1+ T cell.

In another preferred embodiment, said activated T cell provides B cell help.

30

In still another preferred embodiment, said activated T cell induces or otherwise up-

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regulates a TH1 or TH2 response.

In another most preferred embodiment, said GPI comprises a structure selected from:

- 5 EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
- EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y
- EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y
- EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y

10 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 15 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:

- 20 EtN-P-M α 2M α 6M α 4G-Y
- M α 2M α 6M α 4G-Y
- EtN-P-M α 2M α 6M-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 25 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y
 30 is any lipid or phospholipid.

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In still another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 10 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15 In yet another preferred embodiment, said GPI comprises a structure selected from:



20

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 25 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another preferred embodiment, said GPI comprises a structure selected from:

30



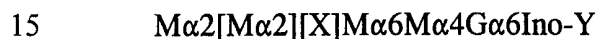
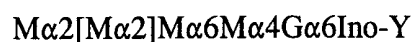
- 39 -



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 5 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

10

In a further embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 20 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

25 In another further preferred embodiment, said GPI comprises a structure selected from:



30

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is

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mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 5 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still another further preferred embodiment, said GPI comprises a structure selected from:

10



15 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 20 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another further preferred embodiment, said GPI comprises a structure selected from:

25



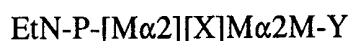
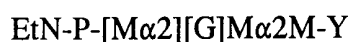
or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 30 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or

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inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

5

In another preferred embodiment, said GPI comprises a structure selected from:



10



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
15 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

20 Accordingly, another aspect of the present invention contemplates a method of treating a mammal said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

25

In one preferred embodiment said mammal has a parasitic infection and more preferably a *Plasmodium* infection. Most preferably said activated helper T cells provide B cell help.

Still more preferably said GPI is *Plasmodium* GPI.

30

In another preferred embodiment, said mammal is in need of the induction, modulation or

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skewing of a helper T cell response along the TH1-or TH2 pathway and said activated T helper cells induce, modulate or skew the T helper response along the TH1 or TH2 pathway.

- 5 In another aspect the present invention contemplates a method of treating a mammal said method comprising administering a mammalian helper T cell activation effective amount of a GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

10

In one preferred embodiment said mammal has a parasitic infection and more preferably a *Plasmodium* infection. Most preferably said activated helper T cells provide B cell help.

Still more preferably said GPI is *Plasmodium* GPI.

15

In another preferred embodiment, said mammal is in need of the induction, modulation or skewing of a helper T cell response along the TH1 or TH2 pathway and said activated T helper cells induce, modulate or skew the T helper response along the TH1 or TH2 pathway.

20

Yet another aspect of the present invention contemplates the use of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell, in the manufacture of a medicament for the activation of helper T cells in a mammal.

25

In one preferred embodiment said mammal has a parasitic infection and more preferably a *Plasmodium* infection. Most preferably said activated helper T cells provide B cell help.

Still more preferably said GPI is *Plasmodium* GPI.

30

In another preferred embodiment, said mammal is in need of the induction, modulation or

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skewing of a helper T cell response along the TH1 or TH2 pathway and said activated T helper cells induce, modulate or skew the T helper response along the TH1 or TH2 pathway.

- 5 The term "mammal" includes humans, primates, livestock animals (e.g. horses, cattle, sheep, pigs and donkeys) laboratory test animals (e.g. mice, rats, rabbits, guinea pigs) companion animals (e.g. dogs and cats) and captive wild animals (e.g. kangaroos, deer, foxes). Preferably, the mammal is a human or laboratory test animal. Even more preferably the mammal is a human.

10

In a related aspect of the present invention the mammal undergoing treatment may be human or an animal in need of therapeutic or prophylactic treatment.

- Accordingly, the present invention provides a method for the treatment and/or prophylaxis
15 of a mammalian disease condition characterised by a micro-organism infection, said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

20

Preferably said microorganism infection is a parasitic infection and more preferably a *Plasmodium* infection. Still more preferably said activated T cells provide B cell help.

- In another aspect, the present invention provides a method for the treatment and/or
25 prophylaxis of a mammalian disease condition characterised by the insufficiency or absence of an appropriate TH1 response said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association induces
30 or otherwise upregulates a TH1 response.

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In still another aspect, the present invention provides a method for the treatment and/or prophylaxis of a mammalian disease condition characterised by the insufficiency or absence of an appropriate TH2 response said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said
5 GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association induces or otherwise upregulates a TH2 response.

Reference to a disease condition "characterised by the insufficiency or absence of an
10 appropriate" TH1 or TH2 response should be understood as a reference to a disease condition in which the appropriate response is either not functional or else is functional at a level too low to be therapeutically or prophylactically effective. It should also be understood as a reference to a condition where one form of helper T cell response is in effect but where it is desirable to skew the response to the other form (for example,
15 skewing a TH1 response to a TH2 response or vice versa). The skewing of the helper T cell response is particularly useful in disease conditions where one or more of the symptoms exhibited by a patient are due to the functional features characteristic of a TH1 or TH2 response, for example the cytokine profile.

20 An "effective amount" means an amount necessary at least partly to attain the desired immune response, or to prevent or to delay the onset or inhibit progression or halt altogether, the onset or progression of a particular condition being treated. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune
25 system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

30 Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a mammal is treated until

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total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered
5 as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition or the frequency of acute attacks (for example, reducing the severity of initial infection).

In accordance with these methods, the GPI or GPI complex defined in accordance with the
10 present invention may be coadministered with one or more other compounds or molecules. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the two types of
15 molecules. These molecules may be administered in any order.

The present invention should also be understood to extend to immunogenic compositions for use in the methods as hereinbefore defined.

20 Accordingly, in a related aspect, the present invention is directed to a composition which activates T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

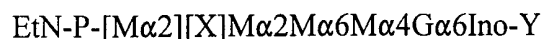
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Preferably said GPI is *Plasmodium* GPI.

In another most preferred embodiment, said GPI comprises a structure selected from:

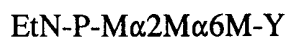
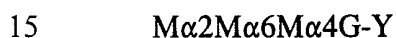
30 EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y

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or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 5 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 10 is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 is any lipid or phospholipid.

25

In still another preferred embodiment, said GPI comprises a structure selected from:



30

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or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In yet another preferred embodiment, said GPI comprises a structure selected from:

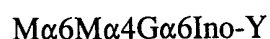
10



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another preferred embodiment, said GPI comprises a structure selected from:

25

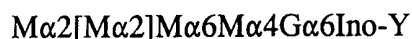


or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may

- 48 -

be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

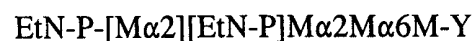
5 In a further embodiment, said GPI comprises a structure selected from:



10

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

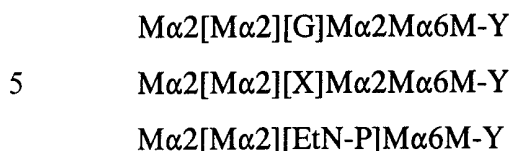
15 In another further preferred embodiment, said GPI comprises a structure selected from:



20 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

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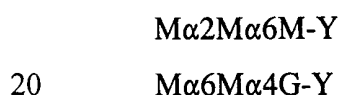
In still another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
10 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15

In still yet another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
25 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

30 In another preferred embodiment, said GPI comprises a structure selected from:

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EtN-P-[M α 2][G]M α 2M-YEtN-P-[M α 2][X]M α 2M-YEtN-P-[M α 2][EtN-P]M α 2M-Y

5 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 10 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another aspect, the present invention relates to an immunogenic composition comprising as the active component GPI or derivative or equivalent thereof or GPI complex, as
 15 broadly described above.

Preferably, said GPI is *Plasmodium* GPI.

In another most preferred embodiment, said GPI comprises a structure selected from:
 20

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-YEtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-YEtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-YEtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y

25

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 30 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y

- 51 -

is any lipid or phospholipid.

In another preferred embodiment, said GPI comprises a structure selected from:

5 EtN-P-M α 2M α 6M α 4G-Y
 M α 2M α 6M α 4G-Y
 EtN-P-M α 2M α 6M-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 10 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 15 is any lipid or phospholipid.

In still another preferred embodiment, said GPI comprises a structure selected from:

EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y
 20 EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is
 mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 25 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 be substituted with β -linkages wherever required, numeric values represent positional
 linkages which may be substituted with any other positional linkages as required, and Y
 is any lipid or phospholipid.

30

In yet another preferred embodiment, said GPI comprises a structure selected from:

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$$M\alpha_2[M\alpha_2][G]M\alpha_2M\alpha_6M\alpha_4G-Y$$

$$M\alpha_2[M\alpha_2][X]M\alpha_2M\alpha_6M\alpha_4G-Y$$

$$M\alpha_2[M\alpha_2][EtN-P]M\alpha_6M\alpha_4G-Y$$

5 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
10 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another preferred embodiment, said GPI comprises a structure selected from:

15 $M\alpha_6M\alpha_4G\alpha_6Ino-Y$

$$M\alpha_2M\alpha_6M\alpha_4G\alpha_6Ino-Y$$

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

25

In a further embodiment, said GPI comprises a structure selected from:

$$M\alpha_2[M\alpha_2]M\alpha_6M\alpha_4G\alpha_6Ino-Y$$

$$M\alpha_2[M\alpha_2][G]M\alpha_6M\alpha_4G\alpha_6Ino-Y$$

30 $M\alpha_2[M\alpha_2][X]M\alpha_6M\alpha_4G\alpha_6Ino-Y$

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or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
 5 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another further preferred embodiment, said GPI comprises a structure selected from:

10



15 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
 20 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still another further preferred embodiment, said GPI comprises a structure selected from:

25



30 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine

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including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y
5 is any lipid or phospholipid.

In still yet another further preferred embodiment, said GPI comprises a structure selected from:

10 $M\alpha_2M\alpha_6M-Y$
 $M\alpha_6M\alpha_4G-Y$

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
15 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

20

In another preferred embodiment, said GPI comprises a structure selected from:

$EtN-P-[M\alpha_2][G]M\alpha_2M-Y$
 $EtN-P-[M\alpha_2][X]M\alpha_2M-Y$
25 $EtN-P-[M\alpha_2][EtN-P]M\alpha_2M-Y$

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
30 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional

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linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

Still another aspect of the present invention is directed to a pharmaceutical composition capable of activating T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1, which association activates helper T cells, together with one or more pharmaceutically acceptable carriers and/or diluents.

10

Preferably, said GPI is *Plasmodium* GPI.

In another most preferred embodiment, said GPI comprises a structure selected from:

15

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y

EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y

20 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
25 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

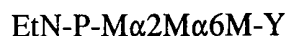
In another preferred embodiment, said GPI comprises a structure selected from:

30

EtN-P-M α 2M α 6M α 4G-Y

M α 2M α 6M α 4G-Y

- 56 -



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 5 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

10

In still another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine
 20 including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

25 In yet another preferred embodiment, said GPI comprises a structure selected from:



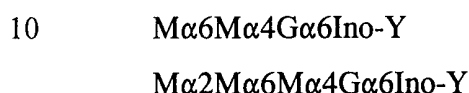
30

or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is

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mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

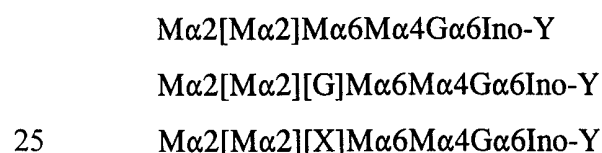
In still yet another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

20

In a further embodiment, said GPI comprises a structure selected from:



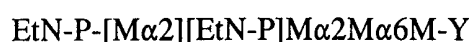
or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional

- 58 -

linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In another further preferred embodiment, said GPI comprises a structure selected from:

5



10 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
15 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

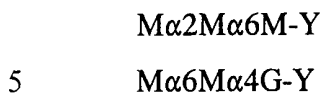
In still another further preferred embodiment, said GPI comprises a structure selected from:

20



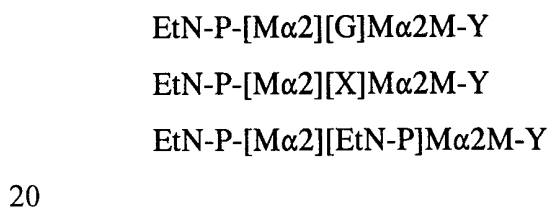
25 or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional
30 linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

In still yet another further preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or
10 inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

15 In another preferred embodiment, said GPI comprises a structure selected from:



or derivative or equivalent thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may
25 be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions
30 (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable

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for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can
5 be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid,
10 thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

15 Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those
20 enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

25 When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible
30 tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active

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compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the
5 present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium
10 phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may
15 be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically
20 pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption
25 delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

30

It is especially advantageous to formulate parenteral compositions in dosage unit form for

ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

10

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μ g to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

20 The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of expressing, for example, a functional equivalent to a GPI or derivative thereof. The vector may, for example, be a viral vector and it may be administered by any suitable method including, for example transfection directly into the cells of the mammal being treated or transfection into a host cell, such as a bacterium, yeast or attenuated parasite, which is then introduced into the mammal.

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The present invention is further described by the following non-limiting Examples:

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EXAMPLE 1

REAGENTS AND ANIMALS

Pronase was obtained from Boehringer Mannheim. Octyl-Sepharose, Protein-G
5 Sepharose, *n*-octylthioglucopyranoside (*n*-otg), phenylmethanesulfonylfluoride (PMSF),
p-tosyl-L-lysinechloromethylketone (TLCK), N-tosyl-L-phenylalaninechloro-
methylketone (TPCK), *p*-chloromercuriphenylsulphonic acid (*p*-CMPS), aprotinin,
leupeptin, pepstatin, iodoacetamide, iodoacetic acid and *n*-ethylmaleimide (NEM) were
obtained from Sigma Chemical Co. Sephadex was from Pharmacia. Analytical or
10 HPLC grade, acetic acid, butanol, chloroform, diethyl ether, ethanol, methanol and
water were obtained from BDH and Waters. Silica G60 TLC plates were from Merck
Darmstadt. Tritiated mannose, glucosamine, myristic and palmitic acids were from
Amersham. The recombinant *P. falciparum* CS protein 2.3 consists of the entire gene
except for the C-terminal 21 amino acids. The *P. berghei* rCS encompasses amino
15 acids 81-277, including the central repetitive domain. The NANP₄₀ peptide, and the
17-mer peptide of the tandemly repeating domain of the *P. berghei* CS protein
(DPPPPNPN)₂D, were synthesized by routine methods.

Adult female C57Bl/6 wild-type mice, C57Bl/6 lacking the MHC Class II gene, or the
20 CD1.1 and CD1.2 genes, congenic mice on the C57Bl/10 or Balb background, Balb/c
nu/nu mice and other mouse haplotypes used in the study were maintained in specific
pathogen free animal facilities.

EXAMPLE 2

CHIMERAS

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Bone marrow was derived from the femurs and tibiae of Balb/c, Balb/B, Balb/K
donors. T cells and NK cells were depleted by complement-mediated lysis of Thy-1⁺,
CD4⁺, Lyt.2⁺ and asialo-GM⁺ cells. Bone marrow aspirates in RPMI 1640 + 3%
30 BSA at 10⁷ cells/ml were incubated on ice with the appropriate dilution of specific
antibody, followed by centrifugation at 2°C and resuspension in low toxicity, mouse

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lymphocyte absorbed, sterile-filtered Guinea pig complement at 37°C in RPMI 1640 + 3% BSA for 1 hr. Syngeneic or allogeneic recipients, maintained on acidified water and tetracycline, were irradiated by cobalt source (1000 Rads) and inoculated i.v. with 10⁷ T-depleted cells. Animals were maintained on acidified water and rested for 12 weeks prior to testing for chimerism and use in experiments. To generate thymic chimeras, thymic lobes were obtained by dissection from neonatal Balb/c, Balb/B, and Balb/K mice and implanted into the interscapular dorsum of adult Balb/c *nu/nu* mice via a subcutaneous incision. Mice were maintained in sterile isolators on acidified water and rested 12 weeks prior to testing for chimerism and use in experiments.

10

EXAMPLE 3

PREPARATION OF BLOOD-STAGE *P. FALCIPARUM* PARASITES

The FCB-1 line of *Plasmodium falciparum* were grown *in vitro* by the method of Trager and Jensen. Synchronous development of parasites was maintained by the sorbitol method of Lambros and Vanderberg. For the biosynthetic labelling of parasite proteins, ³H-palmitic acid conjugated to defatted bovine serum albumin in molar ratio 1:1, ³H-glucosamine or ³H-mannose were added at a final specific activity of 10mCurie/ml, to RPMI 1640 cultures of 2x10¹⁰ parasites at the late trophozoite/early schizont stage for 2 hours (for labelling of GPI precursors) or 8 hours (for labelling of protein-bound GPI). Parasites were harvested by 0.05% Saponin lysis and centrifugation in the cold at 15,000g for 20 minutes, followed by two washes in PBS and storage at -70°C.

25

EXAMPLE 4

PURIFICATION OF THE 195KD MSP-1 AND 56KD MSP-2 ANTIGENS

The GPI-anchored MSP-1 and MSP-2 merozoite surface proteins were purified to homogeneity. Biosynthetically labelled malaria parasites at the late schizont stage were lysed in 0.05% Saponin and centrifuged at 15,000g for 20 minutes, and washed as above. The pellet was extracted in 25mM *n*-octyl-thioglucopyranoside (*n*-otg), 1%

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BSA, 1mM EDTA, 0.1mM EGTA, 1mM PMSF, 1mM TPCK, 0.1mM TLCK, 5mM pCMPS, 1mg/ml pepstatin, 1mg/ml leupeptin, 1mM NEM, 5mM iodoacetamide, 150mM NaCl, 25mM Tris/HCl pH 7.4 by sonication on ice. The extract was clarified by centrifugation at 20,000g for 30 minutes in the cold, and the supernatant decanted and loaded onto two immunoaffinity columns arranged in sequence, containing approximately 10mg monoclonal antibody 111.4 or monoclonal antibody 113.1, each cross-linked to Protein G-Sepharose by glutaraldehyde (all procedures on ice). The protein extract was passed through the column at a rate of 0.3ml/min. The columns were washed first with 100ml 10mM n-otg, 1% BSA, 300mM NaCl, followed by 100ml 10mM n-otg, 300mM NaCl. Antigen was eluted from each column with four column volumes of 10mM n-otg, 200mM glycine pH 2.8. The pH of the eluate was neutralized with 2M Tris. Aliquots of protein were analysed for purity by SDS-PAGE followed by staining with Coomassie brilliant blue. The remaining purified proteins were dialysed exhaustively against 100mM NH_4HCO_3 using dialysis membrane previously boiled exhaustively in 10mM EDTA followed by boiling in 10 changes of double distilled water. Protein concentration was determined by the method of Bradford.

EXAMPLE 5

20 PURIFICATION OF MEMBRANE-FORM VARIANT SURFACE GLYCOPROTEIN (MF VSG) OF *T. BRUCEI*

T. brucei 118 (MITAT 1.5) was purified from the blood of infected Wistar rats by DEAE chromatography. 1×10^{11} parasites were pre-incubated for 30 minutes in glucose-deficient RPMI 1640 supplemented with 40mM fructose and then labelled in the same medium for 90 minutes either with [^3H]-myristic acid conjugated to defatted BSA, or with [^3H]-glucosamine. In the latter case the medium contained 1mg/ml tunicamycin. Parasites were washed in cold medium without label, and taken up in 10mM ZnCl_2 , followed by centrifugation at 45,000g. The pellet was put into boiling 25mM n-otg, 5mM p-CMPS, 1mM PMSF, 1mM TLCK, 50mM Tris/HCL and allowed to cool, and 30 centrifuged at 45,000g at 2°C. The remaining detergent soluble extract was made up to

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1mM CaCl₂, 1mM MgCl₂, and 1mM MnCl₂, and passed over a Con-A sepharose column, followed by washing with 10 column volumes of extraction buffer. The column was first eluted with detergent buffer containing 0.5M α-methyl-mannopyranoside and 0.5M α-glucopyranoside, followed by 25mM *n*-otg in 8M urea.

5 Aliquots were subject to SDS-PAGE and fluorography or staining with Coomassie blue.

EXAMPLE 6

PURIFICATION OF THE C-TERMINAL GPI ANCHORS OF DEFINED PARASITE ANTIGENS

10

GPI-anchored *P. falciparum* MSP-1, MSP-2 and *T. brucei* 118 (MITat 1.5) mfVSG were labelled with fatty acid or glucosamine as required and purified as above, to 10mg/ml. 600ml methanol was added to 150ml aliquots followed by 150ml CHCl₃ and 450ml H₂O. The samples were vortexed and microfuged, the supernatant taken for

15 scintillation counting, and the interphase and lower phase mixed with 450ml methanol and re-centrifuged. The pellet was repeatedly extracted with CMW 10:10:3 until partitioning of fatty-acid label into the supernatant was minimal, partitioned between water and water-saturated butanol, precipitated with acetone at -20°C, and the proteins taken up by sonication in 6M Urea, 1mM DTT, 1mM iodoacetic acid. After 15

20 minutes at room temperature, the sample was diluted 6 fold and made to 5mM CaCl₂. 2.5% pre-digested Pronase B was added, and incubated for 72h at 37°C with 2 additions of 0.25% pronase. The sample was loaded in 5% 1-propanol, 0.1M NH₄OAc onto pre-equilibrated Octyl-Sepharose, washed and eluted in a linear gradient of 1-propanol (5-60%) in water. GPIs eluted at 35-40% 1-propanol and were spotted onto
25 TLC plates (Si-60) and run in the solvent system C/M/HAc/W 25:15:4:2. The origin was scraped, GPIs eluted and partitioned between water and water-saturated butanol.

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EXAMPLE 7**PURIFICATION OF GIPLS AND GPI PRECURSORS BY TLC**

2x10³[H]-glucosamine-labelled *P. falciparum* schizonts were extracted in CM (2:1) and
5 CMW (1:1:0.3), Folch washed, partitioned between water and water-saturated butanol,
and dried. Residues were separated by 2D TLC (1st dimension CMW 4:4:1, 2nd
dimension Butanol/HAc/W 4:6:1), the plates scanned by Bertold Digital
Autoradiograph scanner, and the structurally defined GPI peaks scraped and re-
extracted. Phospholipids were resolved away from GPI peaks. Areas lying outside the
10 identifiable GPI peaks were treated in the same way, as were sham plates. GIPLs of *L.*
mexicana were purified to homogeneity. Briefly, promastigotes were extracted twice in
CMW (1:2:0.8), the insoluble material removed by centrifugation, and the CMW phase
partitioned with 0.6 volume water. The dried upper phase was chromatographed on
Octyl-Sepharose as above, and eluted GIPLs further purified by HPTLC using CM/1N
15 NH₄OH (10:10:3), and scrapings extracted with CMW (1:2:0.8). GIPL concentration
and compositional purity was determined by GC-MS, following acid methanolysis and
trimethylsilyl (TMS) derivatization. *myo*-Inositol content was measured following acid
hydrolysis (6N HCl, 110°C, 16 h) and TMS derivatization, with selected ion
monitoring for *m/z* 305 and 318. *scyllo*-Inositol was used as internal standard
20 throughout.

EXAMPLE 8**GENERATION OF CHEMICAL AND ENZYMATIC HYDROLYSIS
FRAGMENTS OF GPIS AND GIPLS**

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Purified, glucosamine-labelled *P. falciparum* and *T. brucei* GPIs, in which all dpms
were detected in the organic phase following butanol/water partitioning, were subject to
base hydrolysis by suspension in methanol/ammonia 1:1 for 6 hours at 50°C, followed
by partitioning between water and water saturated butanol. Essentially 100% of label
30 was then recovered from the aqueous phase. The aqueous phase was twice extracted
with water-saturated butanol, lyophilized, and flash evaporated with methanol.

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EXAMPLE 9**DEAE ANION EXCHANGE CHROMATOGRAPHY**

GPIs were loaded onto a A DEAE column in 99% methanol, 1% water and washed
5 with ten column volumes of solvent. They were subsequently eluted in 100mM
Ammonium Acetate in 99% methanol, 1% water and dried under Nitrogen.

EXAMPLE 10**BIOGEL P4 SIZE-EXCLUSION CHROMATOGRAPHY**

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Base-hydrolysed GPI glycans were spiked with phenol red and blue dextran in 100mM
Ammonium Acetate and further size-fractionated by passage through a 1cm x 1.2 metre
Biogel P4 column equilibrated in 100mM Ammonium acetate in water. The column
had previously been exhaustively calibrated by repeated analytical runs with GPI mixed
15 with acid hydrolysed dextran markers to yield the relative elution position of glucose
units detected by staining with orcinol in concentrated sulfuric acid. The column runs
proved to be highly reproducible. For preparative purposes the dextran markers were
omitted. The GPI peak was detected by scintillation counting of aliquots. Glycan
concentration and compositional purity was determined by GC-MS, following acid
20 methanolysis and trimethylsilyl (TMS) derivatization. *myo*-Inositol content was
measured following acid hydrolysis (6N HCl, 110°C, 16 h) and TMS derivatization,
with selected ion monitoring for *m/z* 305 and 318. *scyllo*-Inositol was used as internal
standard throughout.

25

EXAMPLE 11**COUPLING OF GPI GLYCAN TO MALEIMIDE-ACTIVATED PROTEINS**

GPIs were coupled to proteins by two methods. (i) GPIs were exposed to 1mM Traut's
reagent (2-iminothiolane) in 40% 1-propanol, 60mM triethanolamine, 7mM potassium
30 phosphate, 100mM NaCl, 1mM EDTA, pH 8.0 in the cold for 90 minutes under
nitrogen. The sample was then desalted by gel filtration at 4°C through a small

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immobilized dextran desalting column equilibrated in 40% 1-propanol in water. The propanol was dried off under nitrogen and the sample added to maleimide-activated KLH (Pierce) in coupling buffer (7mM potassium phosphate, 100mM NaCl, 1mM EDTA, pH 7.2) overnight followed by quenching with cysteine. (ii) (i) GPIs were
5 exposed to 1mM Traut's reagent (2-iminothiolane) in 60mM triethanolamine, 30mM n-otg, 7mM potassium phosphate, 100mM NaCl, 1mM EDTA, pH 8.0 in the cold for 90 minutes under nitrogen. The sample was then desalted by gel filtration at 4°C through a small Biogel P4 column equilibrated in 30mM n-otg, 7mM potassium phosphate, 100mM NaCl, 1mM EDTA, pH 7.2 and added to maleimide-activated KLH (Pierce) in
10 coupling buffer (7mM potassium phosphate, 100mM NaCl, 1mM EDTA, pH 7.2) overnight. The degree of conjugation was estimated by both by comparison of cpms before and after dialysis of the sample against PBS, or by use of Ellmans reagent to quantify sulfhydryl groups. Excess reactive groups were quenched with cysteine.

15

EXAMPLE 12

FACS ANALYSIS

2x10⁵ cells in ice cold murine tonicity RPMI 1640 with 0.05% Sodium azide and 1% BSA were incubated with optimally titrated FITC-, biotin- or phycoerythrin-conjugated
20 monoclonal antibodies to murine CD3, CD4, CD5, CD8, CD25, CD28, CTLA-4, CD44, CD69, Thy-1 (NIMR-1 or G7), and NK1.1 as required. After washing in the same medium the cells were counter-stained with 0.5mg/ml propidium iodide and analysed by FACScan. Cells were sorted on FACStar II.

25

EXAMPLE 13

CYTOKINE CAPTURE ELISA ASSAYS

Cytokine levels were determined by specific capture ELISAs (Pharmingen). Treated and control samples were incubated at 37°C for 2 h in 96-well plates precoated with
30 monoclonal anti-mouse IFN-g, IL-2 and IL-4, followed by washing and probing under the same experimental conditions with biotinylated second antibody anti-mouse IFN-g,

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IL-2 and IL-4, followed by streptavidin-peroxidase. After addition of the substrate and color development, the plates were read by Titertek Multiscan MCC/340 automated ELISA reader. Cytokine mass was calculated by interpolating the results with the standard curve plotted with titrated recombinant mouse IFN- γ , IL-2 and IL-4 of known
5 mass.

EXAMPLE 14

ELISA ASSAY

10 Antigen (tetanus toxoid, *P. falciparum* rCS, *P. berghei* rCS, NANP₄₀-BSA, FLU-BSA or BSA alone) at 10ug/ml in phosphate binding buffer was incubated overnight in 50ul volumes in flat-bottomed Immulon 96-well plates, followed by extensive washing with buffer. The plates were blocked with 1% BSA in PBS for several hours. From a 1/32 dilution, sera were titrated two-fold in 1% BSA in PBS, and 50ul aliquots incubated in
15 triplicate for 2 hours at room temperature, followed by extensive washing with 1% BSA, 0.05% Tween-20 in PBS. An aliquot of affinity purified, biotin-labelled isotype specific goat anti-mouse second antibody was incubated as above, followed by further washing and the addition of streptavidin-alkaline phosphatase. After 30 minutes the plates were washed again and colourimetric development initiated by the addition of p-
20 Nitrophenylphosphate in diethanolamine buffer. Background binding to BSA-coated plates was determined in parallel. The titres derived are the last point giving values statistically different by two-way analysis of variance from non-specific binding by the same serum to the BSA-coated plates.

25

EXAMPLE 15

ANTIBODY FORMATION *IN VITRO*

Donor Balb/c *nu/nu* mice were primed twice with *P. berghei* SPZs or twice with LPS^{FLU}. 10⁵ splenocytes were placed in culture for 7 days in the presence of IL-2, with
30 and without antigen, anti-Class I, anti-Class II and anti-CD1 as indicated, with 10⁴ CD4⁺, NK1.1⁺ cells positively selected by FACS sorting and Dynal Detachabead from

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spleens of C57Bl6 donors primed to GPI-KLH. *P. berghei* SPZs or GPI-OVA^{FLU} were added with or without antibodies to CD1, MHC Class I or MHC Class II at various concentrations and cultured in RPMI 1640 + 10% FCS, 5×10^{-5} M b-ME and left for 8 days. Antigen-specific IgG production in the culture supernatant was determined by
5 ELISA against recCS and fluoresceinated Dog serum albumin (Sigma) as capture antigen.

EXAMPLE 16

MHC II RESTRICTION IN THE ANTIBODY RESPONSE

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This study determined that the antibody response to the native CS protein of malaria sporozoites is not Class II-restricted. Allogeneic bone-marrow irradiation chimeras, demonstrably unable to respond to the protein antigens Tetanus toxoid or recCS, were essentially equal to syngeneic chimeric controls in responding to irradiated sporozoites
15 with the production of anti-CS IgG1 and IgG2 (Table 1). Nude mice cannot respond to sporozoites with anti-CS IgG, and as reported previously (3), passive transfer of cytolytic anti-CD4, but not anti-CD8, antibodies into euthymic animals abrogated anti-CS antibody formation in response to sporozoites. However, nude mice engrafted with irradiated neonatal allogeneic thymi produced anti-CS IgG1 and IgG2 in response to
20 sporozoites at levels similar to recipients of syngeneic thymic implants, but did not respond to recCS or other protein antigens (Table 1). Class II^{0/0} mice, lacking both Class II and Class II-restricted CD4⁺ T cells, and reportedly incapable of mounting IgG responses to T-dependent protein antigens (4-6), responded to native but not recombinant CS protein with high titres of IgG1, IgG2a and IgG2b, similar to wildtype
25 controls (Table 2). Together, these data demonstrate that thymically-derived CD4⁺ cells are required for IgG formation in response to the native CS protein, but that the Class II MHC is not a restriction element in this response.

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EXAMPLE 17

REQUIREMENT FOR GPI LIPID DOMAIN

To determine whether the GPI anchor could account for the difference in immunological behaviour of the native and recombinant proteins, GPIs purified from *Plasmodium falciparum*, *Leishmania mexicana* and *Trypanosoma brucei* were covalently linked in a molar ratio of 1:1 with haptenated Ovalbumin (GPI-OVA^{FLU}). In contrast to sham-OVA^{FLU} alone, GPI-OVA^{FLU} was able to induce anti-FLU and anti-OVA IgG1, IgG2a and IgG2b formation in MHC Class II^{o/o} mice (Table 2). IgG responses in Class II^{o/o} mice were obtained only in response to OVA^{FLU} coupled to intact GPI, and not to OVA^{FLU} coupled to the deacylated GPI glycan, indicating the GPI lipid domain is required, and the glycan not sufficient, for the phenomenon (Table 2). However, GPI-OVA^{FLU} was unable to induce anti-FLU IgG responses in nude mice or euthymic animals treated with lytic anti-CD4 indicating that the GPI does not provide a sufficient signal to drive immunoglobulin class switch in the absence of CD4⁺ cells. Isotype distributions similar to those obtained by immunization of Class II^{o/o} mice with GPI-OVA^{FLU} were observed following exposure to the intact membrane form Variant Surface Glycoprotein of *T. brucei*, but not the deacylated soluble VSG derived by phospholipase C hydrolysis, confirming the requirement for GPI lipid domain and demonstrating that the phenomenon is generalizable to other GPI anchored proteins.

EXAMPLE 18

PROLIFERATIVE AND FUNCTIONAL RESPONSES TO GPI ANCHORED PROTEINS

The *in vitro* proliferative and functional responses to purified GPIs of splenocytes from wild-type and Class II^{o/o} animals primed with either neo-GPI-anchored proteins, *Plasmodium falciparum* or *P. berghei* sporozoites, and blood stage *P. chabaudi* infection were examined. In all cases, compositionally pure GPIs (by GC/MS) elicited proliferative responses from splenocytes. The blastoid cells responding to GPI were predominantly NK1.1⁺, CD4^{int}, TCR α/β ^{int}, V α 14⁺ and CD69⁺, and the FACS

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profiles revealed both a relative and absolute increase in this cell population. This was also accompanied by the production of high levels of IL-4. This unusual population of T cells was considerably expanded following exposure of mice to sporozoites. The proliferative and cytokine response of NK1 + CD4 + T cells to purified GPIs could be
5 blocked by anti-CD1 monoclonal antibody 1B1(7).

EXAMPLE 19

ANTIBODY PRODUCTION

10 When splenocytes from sporozoite primed animals were exposed to sporozoites, they produced measurable levels of anti-CS IgG in the culture supernatant after 8 days. This antibody formation *in vitro* could be substantially blocked by anti-CD1 mAb 1B1, but less so by antibodies to Class II and not at all by anti-Class I. Furthermore, the NK1.1⁺ cells were able to cooperate with B cells in CD1-restricted antibody formation
15 to GPI-OVA^{FLU} but not OVA^{FLU}.

Wild-type and CD1^{o/o} mice were exposed to sporozoites. Approximately 8-16 fold difference in anti-CS end-titres were detected, indicating that antibody formation to the native protein was reduced by approximately 90% in the latter animals, but in response
20 to either TT or recCS was identical to wild-type controls (Table 3).

EXAMPLE 20

COOH-terminal GPI's were purified from affinity-purified GPI-anchored proteins of *P.*
25 *falciparum* (PfGPI) and *Trypanosoma brucei* membrane-form variant surface glycoprotein (mfVSG), and non-protein-linked free GPIs from *Leishmania mexicana*. The compositional purity of these latter molecules was confirmed by gas chromatography-mass spectrometry (GC-MS). In addition, a phosphorylated and lipidated mammalian GPI based on the rat brain Thy-1 GPI, and the corresponding
30 inositolphosphoglycan (IPG) lacking a lipid tail, both chemically synthesized by n-pentenyl glucoside strategy and compositionally pure by ¹H NMR analysis, were also

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used.

NK1.1⁺ CD4⁺ cells from wild-type and class II^{-/-} mice, when cultured with irradiated wild-type or class II^{-/-} antigen-presenting cells (APCs), responded to these purified GPIs as determined by incorporation of [³H]-thymidine ([³H]TdR), and the production of high levels of IL-4 and IFN- γ . No proliferation in the absence of APCs indicated that GPIs do not provide a direct activation signal to NKT cells sufficient to induce cell growth. NKT cells did not respond to GPIs when cultured with irradiated APCs from β_2 M^{-/-} and CD1.1/CD1.2^{-/-} (CD1^{-/-}) donors, or with wild-type and class II^{-/-} APCs in the presence of anti-CD1.1, but responded fully in the presence of isotype controls. The proliferative and IL-4 response to GPI of NKT cells and the V α 14⁺, CD4⁺ subset in unfractionated splenocytes could also be blocked by the anti-CD1 mAb 1B1. Thus the recognition of GPIs by NKT cells is MHC-independent and CD1-restricted.

In addition, NKT cells produced IL-4 in response to CD1.1-transfected J774 macrophages in the absence of exogenous antigen, but not to sham-transfected controls. Nonetheless, the response was enhanced when CD1.1-transfectants were pulsed with GPI. However, in the absence of exogenous antigen no cytokine expression was detected in response to APCs expressing normal level of CD1.1. Thus high levels of CD1.1 expression in transfected cells may alone be sufficient to drive proliferation. Alternatively, as NKT cells can respond to mammalian GPIs (for example, synthetic Thy-1 GPI), CD1.1-transfectants may be able to present endogenous GPIs to NKT cells.

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EXAMPLE 21

Both murine and human cerebral malaria are TH1-driven pro-inflammatory diseases. The *P. berghei* ANKA murine cerebral malaria model has several features in common with the human cerebral malaria syndrome. Specifically, it is a TNF- α and IFN- γ dependent encephalitis associated with upregulation of ICAM-1 on the cerebral microvascular endothelium, an increase in both parasite and macrophage/neutrophil

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adherence to these target cells, and attendant neurological complications. Unlike human cerebral malaria, there is a breakdown of the blood-brain barrier in the terminal stages of the murine syndrome. However, in the proximal stages the murine disease reflects more accurately the inflammatory cascade leading to cerebral involvement in humans. This syndrome manifests most clearly in C57B16 and CBA mice, and is clearly a IFN- γ dependent, TH1 type disease. The TH2-promoting cytokine IL-4 can counter this condition and lack of IL-4 makes the condition worse.

Although susceptible to infection with *P. berghei*, Balb/c mice are usually refractory to the cerebral malaria syndrome (they preferentially manifest a TH2 response). It was observed however that Balb/c CD1.1/CD1.2 gene targeted (knockout) mice showed a high rate of *P. berghei*-induced cerebral malaria (Figure 1). This suggests that a CD1-dependent mechanism protects against cerebral malaria. Furthermore, *in vivo* lysis of NKT cells by passive transfer of lytic monoclonal antibody causes expression of the cerebral syndrome, demonstrating a protective effect against cerebral malaria of NKT cells. Consistent with these observations, it was shown, using intracellular cytokine staining and FACS analysis, that the cytokine profiles of conventional T cells in CD1-knockout mice were heavily biased to the TH1 pole i.e. there was a higher frequency of IFN- γ producing cells (Figure 2). This indicates that CD1-dependent T cells play an important role in regulation of downstream TH1/TH2 differentiation. Thus priming against GPI in a CD1-dependent manner could inhibit the development of cerebral malaria in mice and humans.

EXAMPLE 22

RESULTS

T cell-dependent IgG responses to protein antigens are thought to be exclusively MHC class II- restricted. However, allogeneic bone-marrow irradiation chimeras were similar to syngeneic controls in responding to malaria sporozoites (SPZ) with IgG to the Circumsporozoite (CS) protein, despite inability to respond to the nominal protein antigens tetanus toxoid (TT) or a full-length recombinant *Plasmodium falciparum* CS

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protein (recCS). Nude mice cannot respond to SPZ with anti-CS IgG, and passive transfer of depleting anti-CD4 antibodies into euthymic animals abolishes the anti-CS response to SPZ. However, nude mice engrafted with irradiated neonatal allogeneic thymi mounted anti-CS IgG responses to SPZ similar to recipients of syngeneic thymi, but did not respond to recCS or TT. Mice lacking both class II and class II-restricted CD4⁺ T cells, and unable to respond to T-dependent antigens, produced anti-CS IgG (mean log₂ reciprocal titer of 10) in response to SPZ. Thus CD4⁺ T cells are required for the IgG response to the native CS protein, but this may proceed through a MHC class II-independent route.

10

To determine whether the GPI anchor accounts for the difference in immunological behaviour of the proteins, we purified COOH-terminal GPIs from affinity-purified GPI-anchored proteins of *P. falciparum* (PfGPI) and *Trypanosoma brucei* membrane-form variant surface glycoprotein (mfVSG) (Fig. 3A), and non-protein-linked free GPIs from *Leishmania mexicana* (Fig. 3B). The compositional purity of these latter molecules was confirmed by gas chromatography-mass spectrometry (GC-MS). In addition, a phosphorylated and lipidated mammalian GPI based on the rat brain Thy-1 GPI (Fig. 3C), and the corresponding inositolphosphoglycan (IPG) lacking a lipid tail, both chemically synthesized by *n*-pentenyl glucoside strategy and compositionally pure by ¹H NMR analysis, were also used. To generate responses to the hapten fluorescein (FLU), native and synthetic GPIs and IPG were exposed to 2-iminothiolane to introduce a sulfhydryl onto free amino groups, de-salted and conjugated in a molar ratio of 1:1 to fluoresceinated, maleimide-activated ovalbumin (OVA^{FLU}). In contrast to sham-OVA^{FLU} alone, or sham-OVA^{FLU} mixed with equal molar amounts of free PfGPI, PfGPI-OVA^{FLU} conjugates were able to induce anti-FLU IgG1 formation in MHC class II^{-/-} mice (mean log₂ reciprocal titer of 8). Similar IgG responses were obtained in class II^{-/-} mice with the mfVSG of *T. brucei*, but not the deacylated sVSG derived by PI-PLC hydrolysis (Fig. 3A), demonstrating that the GPI lipid domain is required, and the GPI glycan is not sufficient, for the phenomenon. This was confirmed by comparing responses to OVA^{FLU} conjugated to either synthetic Thy-1 GPI or Thy-1 IPG lacking fatty acid (log₂ reciprocal titer of 9.75 vs. no response respectively). Thus IgG

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responses in class II^{-/-} mice require linkage of antigen to GPI with an intact lipid, which may be of diacylglycerol or alkylacylglycerol composition.

The *in vitro* proliferative and cytokine responses to purified malarial GPI of splenocytes from animals primed with malaria SPZ was examined. There was a marked increase in both the relative and absolute numbers of NK1.1⁺ CD4⁺ blastoid cells responding to GPI from both class II^{-/-} (Fig. 4A) and wild-type mice. A high frequency of both V_α14⁺ CD4⁺ (Fig. 4A) and V_β8⁺ cells was also detected in the responding population. No exogenous cytokines were required for this proliferation, but supplementation of cultures with 5U/ml IL-2 increased the level of response.

To examine the fine specificity of responding cells, splenocytes from wild-type and class II^{-/-} animals primed to *P. berghei* SPZ were exposed to 0.5μM of the structures shown in Fig. 3, plus dipalmitoyl-PI. The cells responded to a similar degree to most intact GPIs, but only weakly to the iM2 GPI with truncated glycan and not at all to glycans lacking the fatty acid domain, or to PI (Fig. 4B). Thus both glycan and fatty acids are required for recognition, and NKT cells from SPZ-primed donors respond to a range of GPIs from diverse protozoal and mammalian taxa. However, as the full range of structures presented to the host under these priming conditions is not known, the results may reflect either broad recognition of diverse antigens by the general population of NKT cells or heterogeneous responses from a clonally mixed population. To analyze this further, cells were expanded in the presence of either PfGPI or Thy-1 GPI for four days, rested in IL-2, then re-stimulated with homologous or heterologous antigen. Cells expanded by either antigen responded significantly less well to the heterologous stimulus (Fig. 4B). Analysis of antigen-specific frequencies following *in vitro* culture revealed no increase above background in response to PfGPI in naïve donors, but a clear increase in antigen-reactive NKT cells from SPZ-primed donors (7-30% of total). However, naïve and primed donors both mounted significant responses to the iM4 GPI (to 5% of NKT cells).

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When sorted NK1.1⁺ CD4⁺ cells from wild-type and class II^{-/-} mice primed to SPZs were cultured with irradiated wild-type or class II^{-/-} antigen-presenting cells (APCs), they responded to purified GPIs as determined by incorporation of [³H]-thymidine ([³H]TdR), and the production of high levels of IL-4 (Fig. 5A). No proliferation in the
5 absence of APCs indicated that GPIs do not provide a direct activation signal to NKT cells sufficient to induce cell growth. NKT cells did not respond to GPIs when cultured with irradiated APCs from $\beta_2M^{-/-}$ and CD1.1/CD1.2^{-/-} (CD1^{-/-}) donors, or with wild-type and class II^{-/-} APCs in the presence of anti-CD1.1 (1B1), but responded fully in the presence of isotype controls (Fig. 5A). The proliferative and IL-4 response to PfGPI
10 of NKT cells and the V α 14⁺, CD4⁺ subset in unfractionated splenocytes could also be blocked by the anti-CD1 mAB 1B1 (Fig. 5B). Thus the recognition of GPIs by NKT cells in MHC-independent and CD1-restricted. In addition, NKT cells produced IL-4 in response to CD1.1-transfected J774 macrophages in the absence of exogenous antigen, but not to sham-transfected controls. Nonetheless, the response was enhanced
15 when CD1.1-transfectants were pulsed with PfGPI (Fig. 5A). The response of NKT cells to CD1.1, observed previously, has been adduced in support of the proposition that this cell population may play a physiological role in the absence of associative recognition of antigen. However, as reported, in the absence of exogenous antigen no cytokine expression was detected in response to APCs expressing normal level of
20 CD1.1. Thus high levels of CD1.1 expression in transfected cells may alone be sufficient to drive proliferation. Alternatively, as NKT cells can respond to mammalian GPIs (for example, synthetic Thy-1 GPI (Fig. 4B)), CD1.1-transfectants may be able to present endogenous GPIs to NKT cells.

25 Extending to antigen-specific systems, NKT cells cooperated with B cells by ELISPOT assay in CD1-restricted IgG formation to GPI-OVA^{FLU} and native *P. berghe* CS protein, but not OVA^{FLU} (Fig. 6A). To determine therefore whether CD1.1 or CD1.2-restricted antibody formation was a major or minor contributor to the IgG response to GPI-anchored proteins and SPZ *in vivo*, CD1^{-/-} mice and wild-type controls were
30 exposed to mfVSG^{FLU}, SPZ or recCS. Responses to mfVSG^{FLU} and SPZ were significantly curtailed in CD1^{-/-} mice (Fig. 6B), indicating that under these conditions

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the CD1-restricted pathway of IgG formation is a significant component of responses to the native CS protein. Both groups responded equally to recCS, confirming that class II-dependent responses are unaffected by loss of CD1.

5

METHODS

Irradiated H-2 congenic mice received 10^7 T-depleted syngeneic or allogeneic bone-marrow cells. Thymic lobes from neonatal Balb/c, Balb/B, and Balb/K mice were irradiated and implanted into adult Balb/c *nu/nu* mice. Mice were rested 12 weeks
 10 prior to testing for chimerism. *P. falciparum* and *P. berghei* SPZ were dissected from *Anopheles freeborni* and *A. stephensi* respectively. Some preparations were kept at -70°C before use. Mice were primed with TT, recCS, or SPZ, boosted after 1 week, and IgG responses analyzed by ELISA. RecCS, derived from the T4 strain and consisting of the entire sequence except for the C-terminal 21 amino acids (GPI-anchor
 15 signal sequence), was used as immunizing antigen and to detect responses to *P. falciparum* SPZs. The *P. berghei* rCS (detection antigen only for *P. berghei* SPZs) encompasses amino acids 81-277. Antigen-specific end-titers were defined as the last titration giving values statistically different from binding to plates without antigen. A $\log_2$ reciprocal titer of 4.0 was the background cutoff. To TT, recCS and *P. berghei*
 20 SPZ, syngeneic thymic chimeras mounted mean $\log_2$ reciprocal IgG titers of 15.14, 13.5 and 13.5, respectively. The equivalent values for allogeneic chimeras were 4.5, 4.25 and 13. For syngeneic bone-marrow chimeras the equivalent values were 15.11, 11.25 and 14.6, and for allogeneic chimeras 8.5, 7.25 and 15.25, respectively. The results were confirmed using *P. falciparum* SPZ.

25

IgG responses to rCS, recCS or FLU were determined as above in class II^{-/-} and wild-type mice receiving *P. berghei* SPZ, recCS, mfVSG^{FLU}, sVSG^{FLU} or OVA^{FLU}, either sham-conjugated (sham-OVA^{FLU}) or conjugated to *P. falciparum* GPI (PfGPI-OVA^{FLU}), Thy-1 GPI (Thy-1-OVA^{FLU}) or Thy-1 IPG (IPG-OVA^{FLU}). Unlike wild-types, Class II^{-/-}
 30 animals failed to raise IgG to recCS, sVSG^{FLU}, sham-OVA^{FLU} and IPG-OVA^{FLU} (mean $\log_2$ reciprocal titers <4). However, they produced IgG in response to SPZ, PfGPI-

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OVA^{FLU}, mfVSG^{FLU} and Thy-1-OVA^{FLU} (log₂ reciprocal titer of 10,8 8.25 and 9.75, respectively). Ig isotypes included IgG1, IgG2a and IgG2b.

- mfVSG and GPI-anchored *P. falciparum* proteins, purified by HPLC and affinity
5 chromatography were extracted in chloroform:methanol, precipitated with acetone,
solubilized and reduced, diluted in 5mM CaCl₂, digested for 72h at 37°C with Pronase
B, purified over Octyl-Sepharose, partitioned between water and water-saturated
butanol (PfGPI), and purified by high performance thin layer chromatography
(HPTLC) ($R_f=0.05$) using C/M/HAc/W (25:15:4:2). CMW (1:2:0.8) extracts of *L.*
10 *mexicana* promastigotes were purified over Octyl-Sepharose followed by HPTLC using
CM/1N NH₄OH (10:10:3). The compositional purity of the latter molecules was
confirmed by GC-MS following acid methanolysis and trimethylsilyl (TMS)
derivatization for neutral monosaccharide analysis, and acid hydrolysis (6N HCl,
110°C, 16 h) and TMS derivatization, with selected ion monitoring for m/z 305 and
15 318. *scyllo*-Inositol was used as internal standard throughout. Compositional molar
ratios were as predicted and no contaminating carbohydrates or sugars were detected.
The specific values are published elsewhere as are the ¹H NMR compositional analyses
demonstrating purity of the synthetic Thy-1 GPI and IPG.
- 20 Those skilled in the art will appreciate that the invention described herein is susceptible
to variations and modifications other than those specifically described. It is to be
understood that the invention includes all such variations and modifications. The
invention also includes all of the steps, features, compositions and compounds referred
to or indicated in this specification, individually or collectively, and any and all
25 combinations of any two or more said steps or features.

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Table 1

MHC Class II is not a restriction element in IgG formation to SPZs.

Test group	Antigen:	TT	recCS	nativeCS
BM chimeras				
Balb/c-----> Balb/c				
	Animal#101	32768	32768	ND
	Animal#103	65536	4096	ND
	Animal#114	32768	8192	ND
	Animal#107	131072	32768	ND
	Animal#106	16384	ND	65536
	Animal#111	32768	ND	32768
	Animal#105	32768	ND	16384
	Animal#121	8192	ND	32768
	Animal#119	65536	ND	8192
Balb/c-----> Balb/K				
	Animal#202	512	64	ND
	Animal#203	128	128	ND
	Animal#205	1024	256	ND
	Animal#207	128	256	ND
	Animal#208	256	ND	32768
	Animal#211	512	ND	16384
	Animal#212	256	ND	65532
	Animal#217	1024	ND	65532
Thymic chimeras				
Balb/c-----> Balb/c				
	Animal#1006	65536	6384	ND
	Animal#1007	16384	16384	ND
	Animal#1014	131072	4096	ND
	Animal#1016	16384	ND	16384
	Animal#1022	32768	ND	8192
	Animal#1025	131072	ND	16384
	Animal#1028	8192	ND	8192
Balb/c-----> Balb/K				
	Animal#2004	32	64	ND
	Animal#2005	16	32	ND
	Animal#2006	< 16	< 16	ND
	Animal#2010	128	< 16	ND
	Animal#2014	32	ND	4096
	Animal#2019	32	ND	16384
	Animal#2020	16	ND	8192
	Animal#2022	< 16	ND	8192

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Table 2.

IgG responses to the recCS, native CS, OVA^{FLU} and GPI-OVA^{FLU}
in C57 wild-type, Class II^{-/-} and β_2 M^{-/-} mice.

Primed with:	Reciprocal titre to:			
	CS		FLU	
	SPZs	recCS	OVA ^{FLU}	GPI-OVA ^{FLU}
1) Wild-type	4096	4096	4096	8192
	4096	8192	1024	512
	4096	8192	2048	512
	16384	16384		< 16
2) Class II ^{-/-}	512	< 16	< 16	128
	512	< 16	< 16	64
	2048	< 16	< 16	512
	2048	32	32	1024
3) β_2 M ^{-/-}	1024	8192	ND	ND
	2048	8192		
	2048	8192		
	4096	8192		

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Table 3.

The IgG response to the native CS protein is reduced in CD1.1^{0/0} mice

Immunized with:	Reciprocal anti-CS titre	
	SPZs	recCS
1) Balb/c	4096	4096
	4096	8192
	8192	8192
	16384	16384
2) Balb/c CD1 ^{0/0}	512	8192
	512	8192
	1024	8192
	2048	8192

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CLAIMS:

1. A method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
2. The method according to claim 1 wherein said helper T cell is a CD4⁺ T cell.
3. The method according to claim 2 wherein said CD4⁺ T cell is a CD4⁺ NK1.1⁺ T cell.
4. The method according to any one of claims 1-3 wherein said complex comprises GPI and malarial CS protein or derivative or equivalent thereof.
5. The method according to any one of claims 1-3 wherein said complex comprises GPI and MSP-1 or derivative or equivalent thereof.
6. The method according to any one of claims 1-3 wherein said complex comprises GPI and MSP-2 or derivative or equivalent thereof.
7. The method according to any one of claims 1-3 wherein said complex comprises GPI and *Leishmanial* PSA-2 or derivative or equivalent thereof.
8. The method according to any one of claims 1-3 wherein said complex comprises GPI and GP63 or derivative or equivalent thereof.
9. The method according to any one of claims 1-8 wherein said GPI is a *Plasmodium* GPI.
10. The method according to claim 9 wherein said *Plasmodium* is *P. falciparum*.

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11. The method according to any one of claims 1-8 wherein said GPI comprises a structure selected from:

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-M α 2M α 6M α 4G-Y
 M α 2M α 6M α 4G-Y
 EtN-P-M α 2M α 6M-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y
 M α 2[M α 2][G]M α 2M α 6M α 4G-Y
 M α 2[M α 2][X]M α 2M α 6M α 4G-Y
 M α 2[M α 2][EtN-P]M α 6M α 4G-Y
 M α 6M α 4G α 6Ino-Y
 M α 2M α 6M α 4G α 6Ino-Y
 M α 2[M α 2]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][G]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][X]M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M-Y
 EtN-P-[M α 2][X]M α 2M α 6M-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M-Y
 M α 2[M α 2][G]M α 2M α 6M-Y
 M α 2[M α 2][X]M α 2M α 6M-Y
 M α 2[M α 2][EtN-P]M α 6M-Y
 M α 2M α 6M-Y
 M α 6M α 4G-Y
 EtN-P-[M α 2][G]M α 2M-Y
 EtN-P-[M α 2][X]M α 2M-Y

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or derivatives or equivalents thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

12. The method according to claim 11 wherein said lipid is diacylglycerol, alkylacylglycerol, monoalkylglycerol, ceramide or sphingolipid.
13. The method according to claim 11 wherein said phospholipid is phosphatidylethanolamine, phosphatidylcholine or phosphatidylserine.
14. A method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells wherein said activated T cells provide B cell help.
15. The method according to claim 14 wherein said helper T cell is a CD4⁺ T cell.
16. The method according to claim 15 wherein said CD4⁺ T cell is a CD4⁺ NK1.1⁺ T cell.
17. A method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells wherein said activated T cells induce or otherwise

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upregulate a TH1 type response.

18. A method of activating helper T cells said method comprising administering a T cell activating effective amount of GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells wherein said activated T cells induce or otherwise upregulate a TH2 type response.
19. The method according to claim 17 or 18 wherein said helper T cell is a CD4⁺ T cell.
20. The method according to claim 19 wherein said CD4⁺ T cell is a CD4⁺, NK1.1⁺ T cell.
21. A method of inducing, in a mammal, an immune response directed to GPI said method comprising administering to said mammal a T cell activating effective amount of GPI or derivative or equivalent thereof which GPI is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
22. The method according to claim 21 wherein said helper T cell is a CD4⁺ cell.
23. The method according to claim 22 wherein said CD4⁺ T cell is a CD4⁺ NK1.1⁺ T cell.
24. The method according to any one of claims 21-23 wherein said GPI is *Plasmodium*.
25. The method according to claim 24 wherein said *Plasmodium* is *P. falciparum*.
26. The method according to any one of claims 21-23 wherein said GPI comprises a structure selected from:

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EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-M α 2M α 6M α 4G-Y
 M α 2M α 6M α 4G-Y
 EtN-P-M α 2M α 6M-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y
 M α 2[M α 2][G]M α 2M α 6M α 4G-Y
 M α 2[M α 2][X]M α 2M α 6M α 4G-Y
 M α 2[M α 2][EtN-P]M α 6M α 4G-Y
 M α 6M α 4G α 6Ino-Y
 M α 2M α 6M α 4G α 6Ino-Y
 M α 2[M α 2]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][G]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][X]M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M-Y
 EtN-P-[M α 2][X]M α 2M α 6M-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M-Y
 M α 2[M α 2][G]M α 2M α 6M-Y
 M α 2[M α 2][X]M α 2M α 6M-Y
 M α 2[M α 2][EtN-P]M α 6M-Y
 M α 2M α 6M-Y
 M α 6M α 4G-Y
 EtN-P-[M α 2][G]M α 2M-Y
 EtN-P-[M α 2][X]M α 2M-Y
 EtN-P-[M α 2][EtN-P]M α 2M-Y

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- or derivatives or equivalents thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.
27. The method according to claim 26 wherein said lipid is diacylglycerol, alkylacylglycerol, monoalkylglycerol, ceramide or sphingolipid.
28. The method according to claim 26 wherein said phospholipid is phosphatidylethanolamine, phosphatidylcholine or phosphatidylserine.
29. A method of inducing, in a mammal, an immune response directed to an antigen, said method comprising administering to said mammal a helper T cell activating effective amount of GPI or derivative or equivalent thereof complexed to said antigen, which GPI-antigen complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
30. The method according to claim 29 wherein said helper T cell is a CD4⁺ T cell.
31. The method according to claim 30 wherein said CD4⁺ T cell is a CD4⁺ NK1.1⁺ T cell.
32. The method according to any one of claims 29-31 wherein said antigen is malarial CS protein or derivative or equivalent thereof.
33. The method according to any one of claims 29-31 wherein said antigen is MSP-1 or derivative or equivalent thereof.

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34. The method according to any one of claims 29-31 wherein said antigen is MSP-2 or derivative or equivalent thereof.
35. The method according to any one of claims 29-31 wherein said antigen is *Leishmanial* PSA-2 or derivative or equivalent thereof.
36. The method according to any one of claims 29-31 wherein said antigen is GP63 or derivative or equivalent thereof.
37. The method according to any one of claims 29-36 wherein said GPI comprises a structure selected from:

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-M α 2M α 6M α 4G-Y
 M α 2M α 6M α 4G-Y
 EtN-P-M α 2M α 6M-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y
 M α 2[M α 2][G]M α 2M α 6M α 4G-Y
 M α 2[M α 2][X]M α 2M α 6M α 4G-Y
 M α 2[M α 2][EtN-P]M α 6M α 4G-Y
 M α 6M α 4G α 6Ino-Y
 M α 2M α 6M α 4G α 6Ino-Y
 M α 2[M α 2]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][G]M α 6M α 4G α 6Ino-Y
 M α 2[M α 2][X]M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M-Y

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EtN-P-[M α 2][X]M α 2M α 6M-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M-Y
 M α 2[M α 2][G]M α 2M α 6M-Y
 M α 2[M α 2][X]M α 2M α 6M-Y
 M α 2[M α 2][EtN-P]M α 6M-Y
 M α 2M α 6M-Y
 M α 6M α 4G-Y
 EtN-P-[M α 2][G]M α 2M-Y
 EtN-P-[M α 2][X]M α 2M-Y
 EtN-P-[M α 2][EtN-P]M α 2M-Y

or derivatives or equivalents thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

38. The method according to claim 37 wherein said lipid is diacylglycerol, alkylacylglycerol, monoalkylglycerol, ceramide or sphingolipid.
39. The method according to claim 37 wherein said phospholipid is phosphatidylethanolamine, phosphatidylcholine or phosphatidylserine.
40. The method according to any one of claims 21-39 wherein said activated helper T cell provides B cell help.
41. The method according to any one of claims 21-39 wherein said activated T cells induce or otherwise upregulate a TH1 type response.

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42. The method according to any one of claims 21-39 wherein said activated T cells induce or otherwise upregulate a TH2 type response.
43. A method for the treatment and/or prophylaxis of a mammalian disease condition comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with the CD1 which association activates helper T cells.
44. The method according to claim 43 wherein said helper T cell is a CD4⁺ T cell.
45. The method according to claim 44 wherein said CD4⁺ T cell is a CD4⁺ NK1.1⁺ T cell.
46. The method according to any one of claims 43-45 wherein said activated T cell provides B cell help.
47. The method according to any one of claims 43-45 wherein said activated T cells induce or otherwise upregulate a TH1 type response.
48. The method according to any one of claims 43-45 wherein said activated T cells induce or otherwise upregulate a TH2 type response.
49. A method for the treatment and/or prophylaxis of a mammalian disease condition characterised by microorganism infection, said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
50. The method according to claim 49 wherein said microorganism infection is a parasitic infection.

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51. The method according to claim 50 wherein said complex comprises GPI and malarial CS protein or derivative or equivalent thereof.
52. The method according to claim 50 wherein said complex comprises GPI and MSP-1 or derivative or equivalent thereof.
53. The method according to claim 50 wherein said complex comprises GPI and MSP-2 or derivative or equivalent thereof.
54. The method according to claim 50 wherein said complex comprises *Leishmanial* PSA-2 or derivative or equivalent thereof.
55. The method according to claim 50 wherein said complex comprises GPI and GP63 or derivative or equivalent thereof.
56. The method according to any one of claims 49-55 wherein said GPI comprises a structure selected from:

EtN-P-[M α 2]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G α 6Ino-Y
 EtN-P-M α 2M α 6M α 4G-Y
 M α 2M α 6M α 4G-Y
 EtN-P-M α 2M α 6M-Y
 EtN-P-[M α 2][G]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][X]M α 2M α 6M α 4G-Y
 EtN-P-[M α 2][EtN-P]M α 2M α 6M α 4G-Y
 M α 2[M α 2][G]M α 2M α 6M α 4G-Y
 M α 2[M α 2][X]M α 2M α 6M α 4G-Y
 M α 2[M α 2][EtN-P]M α 6M α 4G-Y

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$M\alpha_6M\alpha_4G\alpha_6Ino-Y$
 $M\alpha_2M\alpha_6M\alpha_4G\alpha_6Ino-Y$
 $M\alpha_2[M\alpha_2]M\alpha_6M\alpha_4G\alpha_6Ino-Y$
 $M\alpha_2[M\alpha_2][G]M\alpha_6M\alpha_4G\alpha_6Ino-Y$
 $M\alpha_2[M\alpha_2][X]M\alpha_6M\alpha_4G\alpha_6Ino-Y$
 $EtN-P-[M\alpha_2][G]M\alpha_2M\alpha_6M-Y$
 $EtN-P-[M\alpha_2][X]M\alpha_2M\alpha_6M-Y$
 $EtN-P-[M\alpha_2][EtN-P]M\alpha_2M\alpha_6M-Y$
 $M\alpha_2[M\alpha_2][G]M\alpha_2M\alpha_6M-Y$
 $M\alpha_2[M\alpha_2][X]M\alpha_2M\alpha_6M-Y$
 $M\alpha_2[M\alpha_2][EtN-P]M\alpha_6M-Y$
 $M\alpha_2M\alpha_6M-Y$
 $M\alpha_6M\alpha_4G-Y$
 $EtN-P-[M\alpha_2][G]M\alpha_2M-Y$
 $EtN-P-[M\alpha_2][X]M\alpha_2M-Y$
 $EtN-P-[M\alpha_2][EtN-P]M\alpha_2M-Y$

or derivatives or equivalents thereof wherein EtN is ethanolamine, P is phosphate, M is mannose, G is non-N-acetylated glucosamine, [G] is any non-N-acetylated hexosamine including glucosamine, or any other nitrous-acid labile substituent, Ino is inositol or inositol-phosphoglycerol, [X] is any other substituent, α represents α -linkages which may be substituted with β -linkages wherever required, numeric values represent positional linkages which may be substituted with any other positional linkages as required, and Y is any lipid or phospholipid.

57. The method according to claim 56 wherein said lipid is diacylglycerol, alkylacylglycerol, monoalkylglycerol, ceramide or sphingolipid.
58. The method according to claim 56 wherein said phospholipid is phosphatidylethanolamine, phosphatidylcholine or phosphatidylserine.

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59. The method according to claim 49 wherein said parasitic infection is a *Plasmodium* infection.
60. The method according to claim 59 wherein said *Plasmodium* is *P. falciparum*.
61. The method according to claim 49 wherein said parasitic infection is a *Leishmania* infection.
62. A method for the treatment and/or prophylaxis of a mammalian disease condition characterised by the insufficiency or absence of an appropriate TH1 response said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association induces or otherwise upregulates a TH1 response.
63. The method according to claim 62 wherein said disease condition is Leishmaniasis, a neoplastic condition or cancer.
64. A method for the treatment and/or prophylaxis of a mammalian disease condition characterised by the insufficiency or absence of an appropriate TH2 response said method comprising administering to said mammal an effective amount of GPI or derivative or equivalent thereof or a complex comprising said GPI or derivative or equivalent thereof which GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association induces or otherwise upregulates a TH2 response.
65. The method according to claim 64 wherein said disease condition is cerebral malaria, type I diabetes, autoimmune arthritis or systemic lupus erythromatosis.

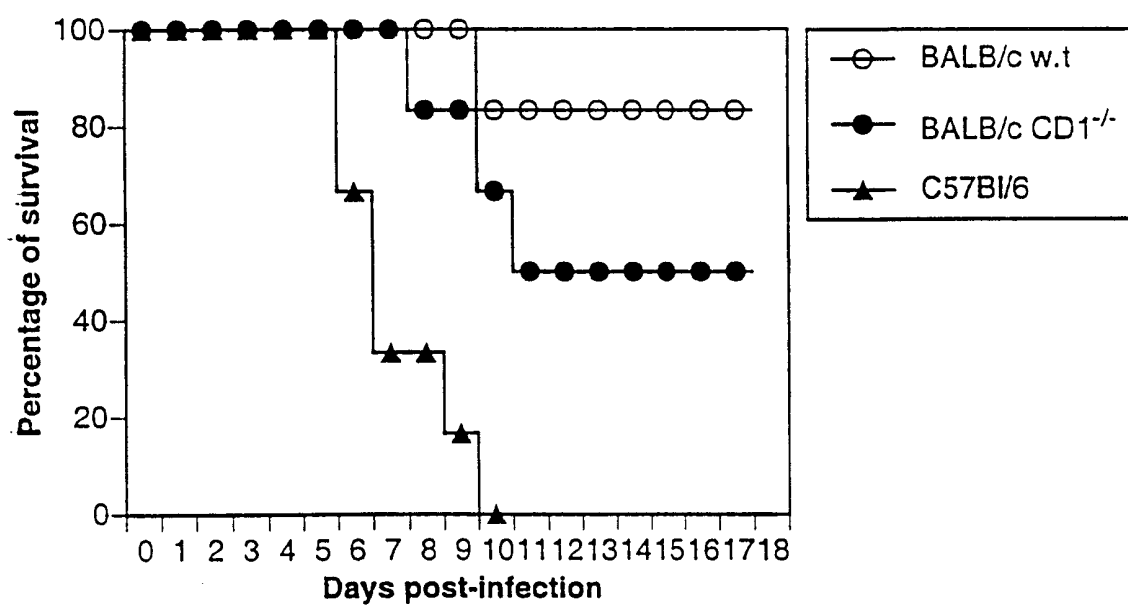
- 97 -

66. Use of a composition comprising GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of a mammalian disease condition wherein said GPI or GPI complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
67. Use according to claim 66 wherein said mammalian disease condition is a microorganism infection.
68. Use according to claim 67 wherein said microorganism is *Plasmodium*.
69. Use according to claim 68 wherein said *Plasmodium* is *P. falciparum*.
70. Use according to claim 67 wherein said microorganism is *Leishmania*.
71. Use according to claim 66 wherein said disease condition is characterised by the insufficiency or absence of an appropriate TH1 response.
72. Use according to claim 71 wherein said disease condition is Leishmaniasis, a neoplastic condition or cancer.
73. Use according to claim 66 wherein said disease condition is characterised by the insufficiency or absence of an appropriate TH2 response.
74. Use according to claim 73 wherein said disease condition is cerebral malaria, type I diabetes, autoimmune arthritis or systemic lupus erythromatosis.
75. A composition capable of activating helper T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.

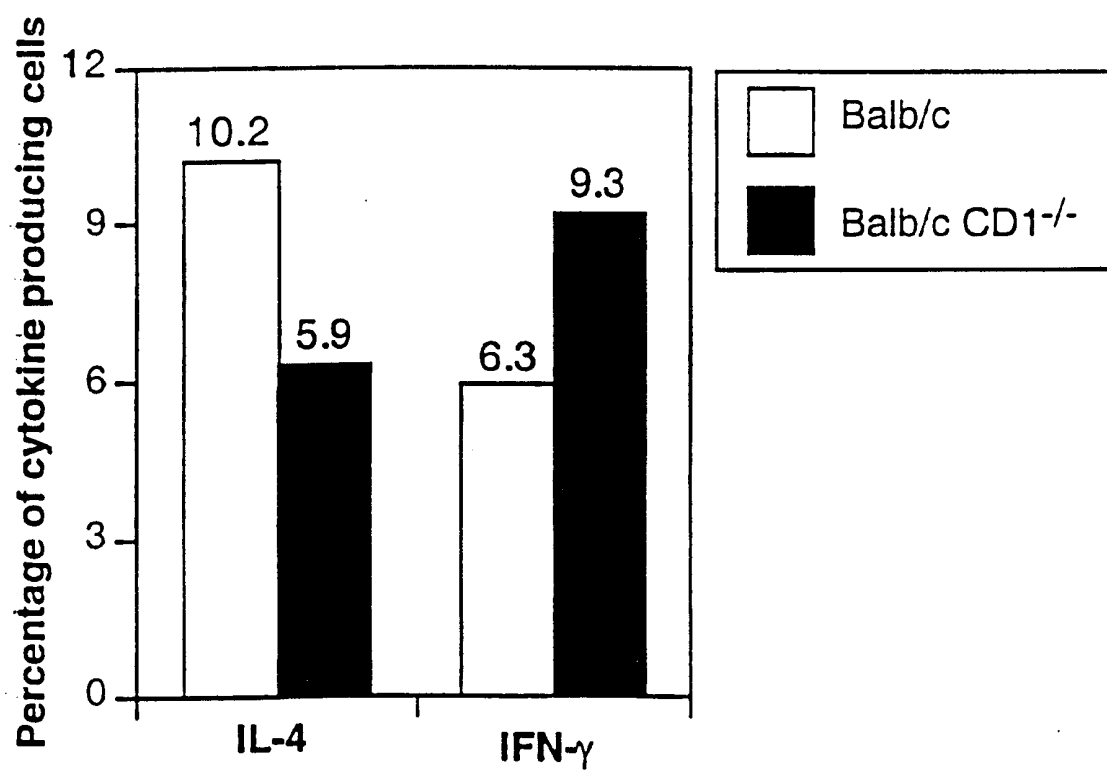
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76. A vaccine composition comprising as the active component a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1 which association activates helper T cells.
77. A pharmaceutical composition capable of activating helper T cells, said composition comprising a GPI or derivative or equivalent thereof or a complex comprising GPI or derivative or equivalent thereof which GPI or GPI-complex is capable of interacting with CD1 on an immune cell to form an association with CD1, which association activates helper T cells, together with one or more pharmaceutically acceptable carriers and/or diluents.

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Parasite protein anchors (PfGPI, mfVSG GPI)

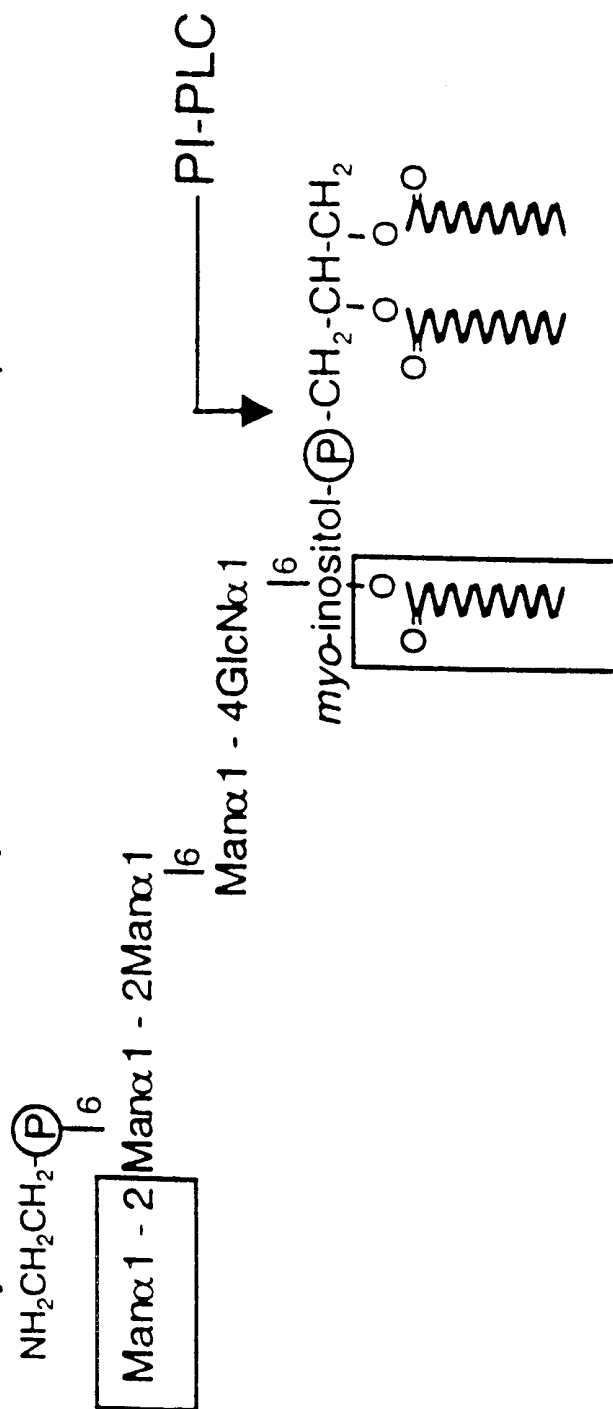


Figure 3a

Leishmanial free GPIs

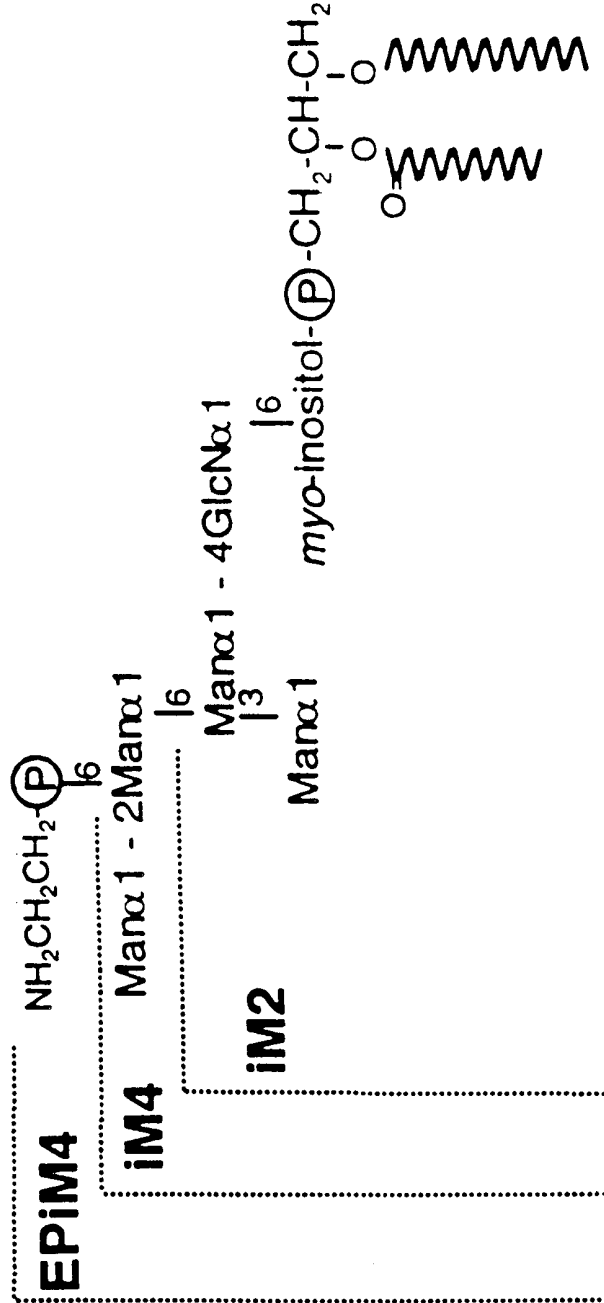


Figure 3b

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Synthetic Thy-1 anchor

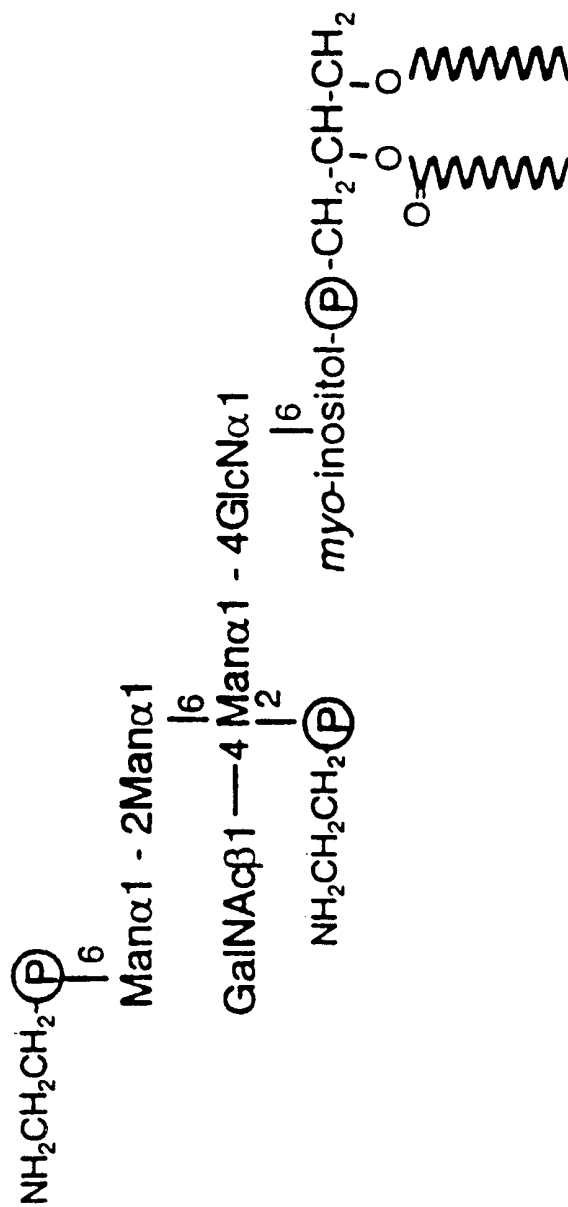


Figure 3c

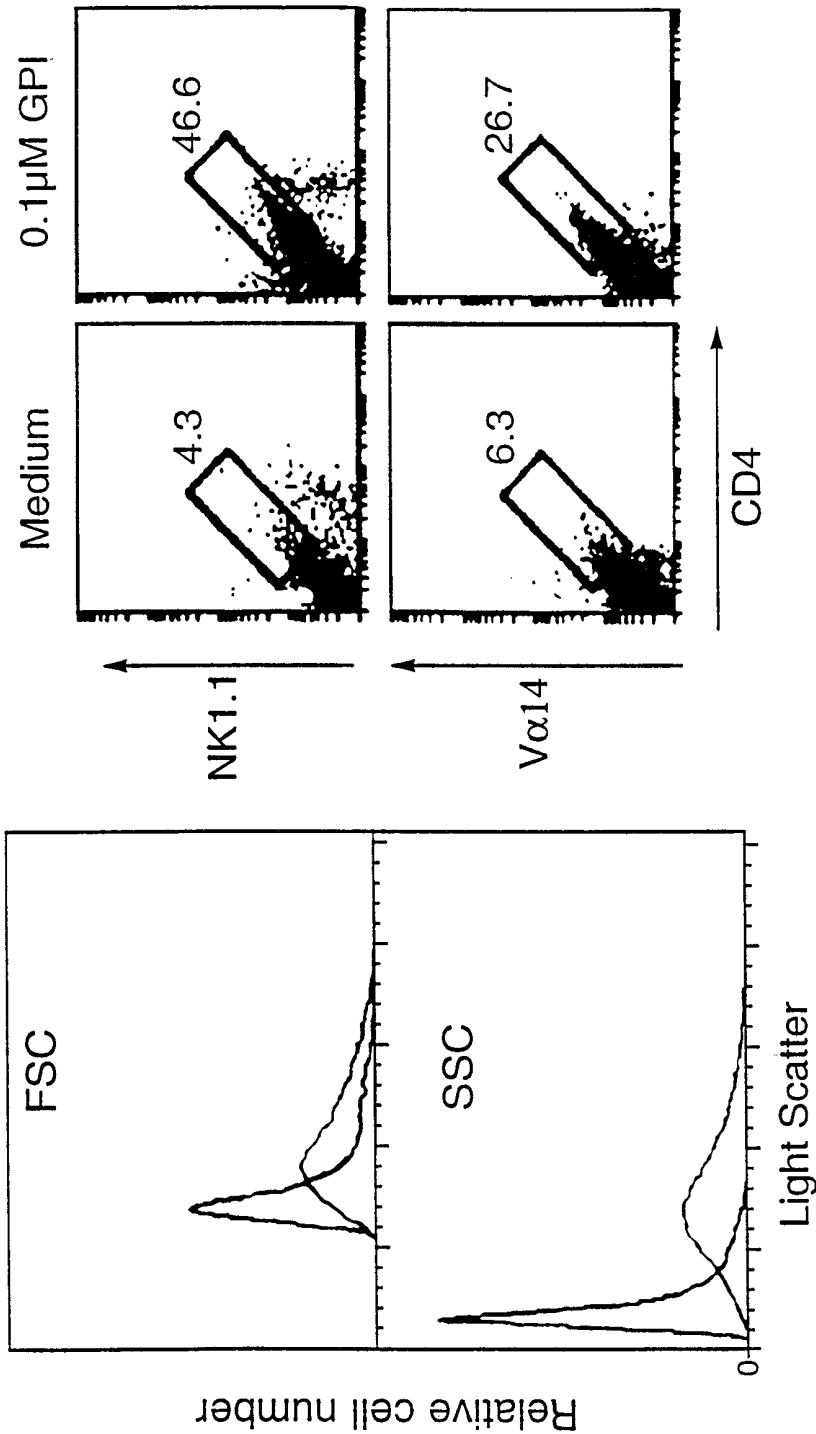


Figure 4a

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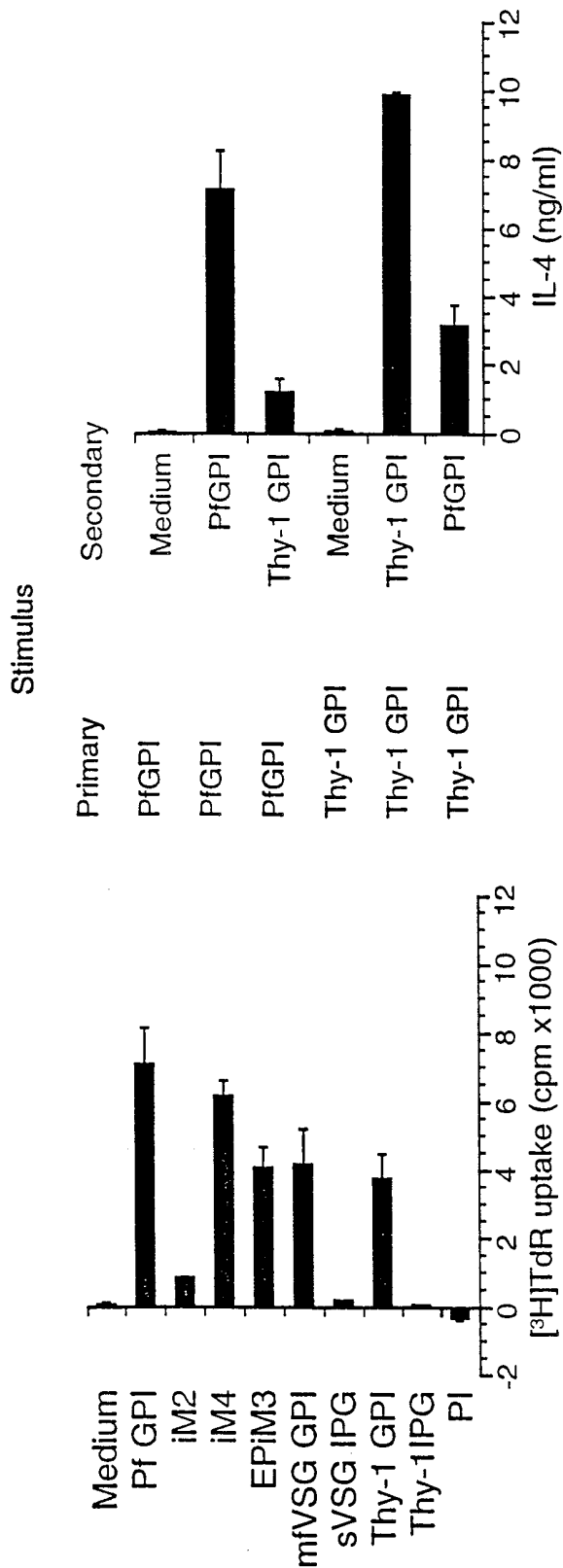
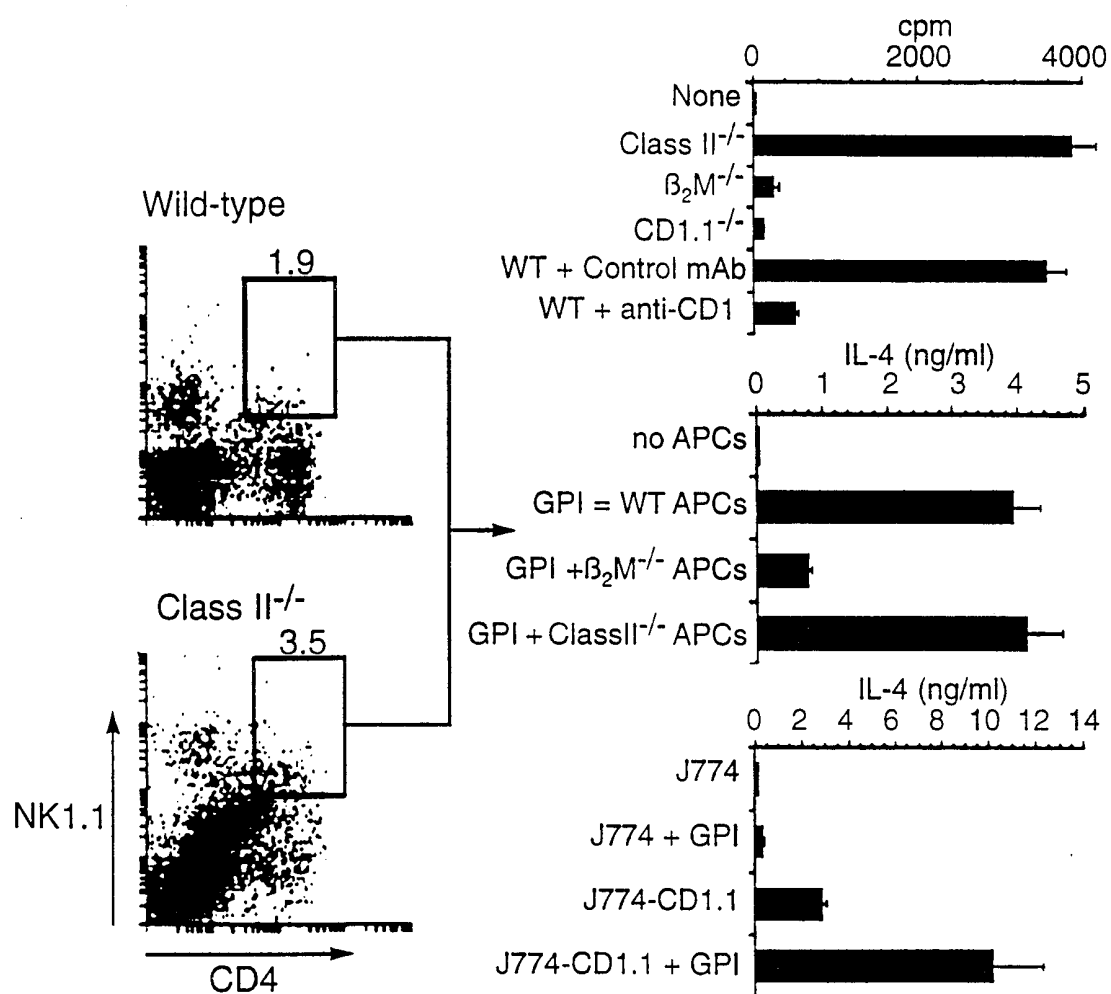
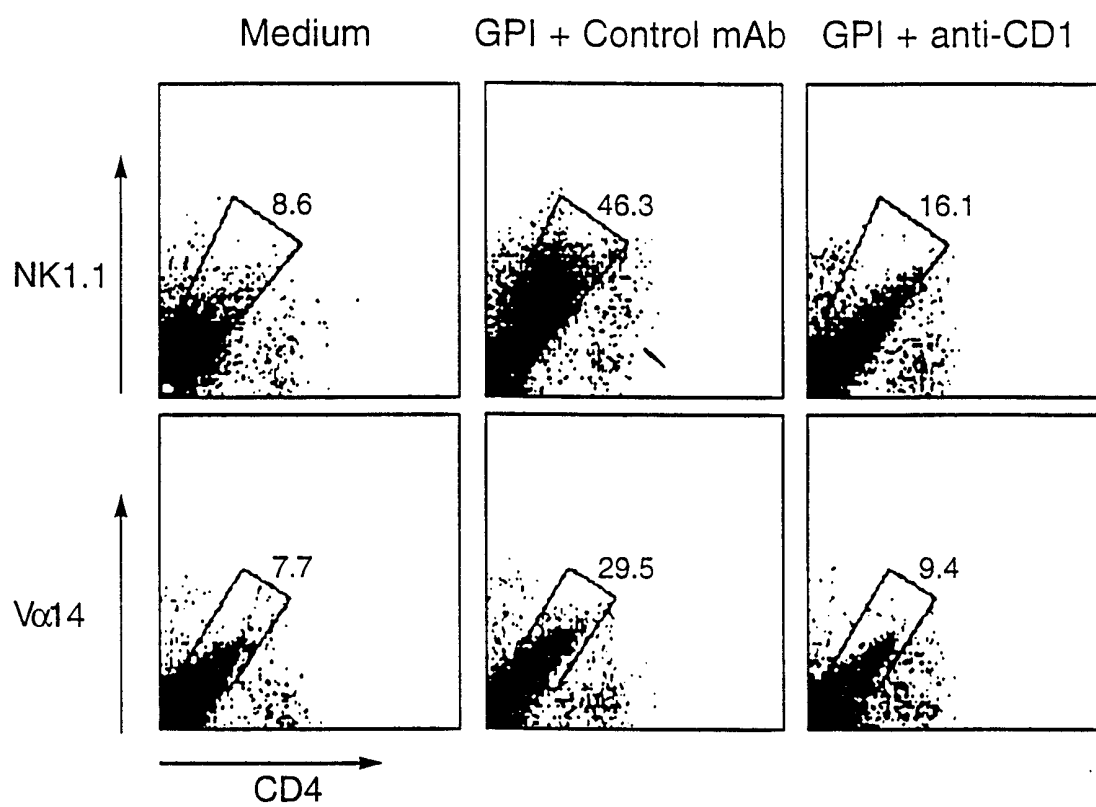


Figure 4b

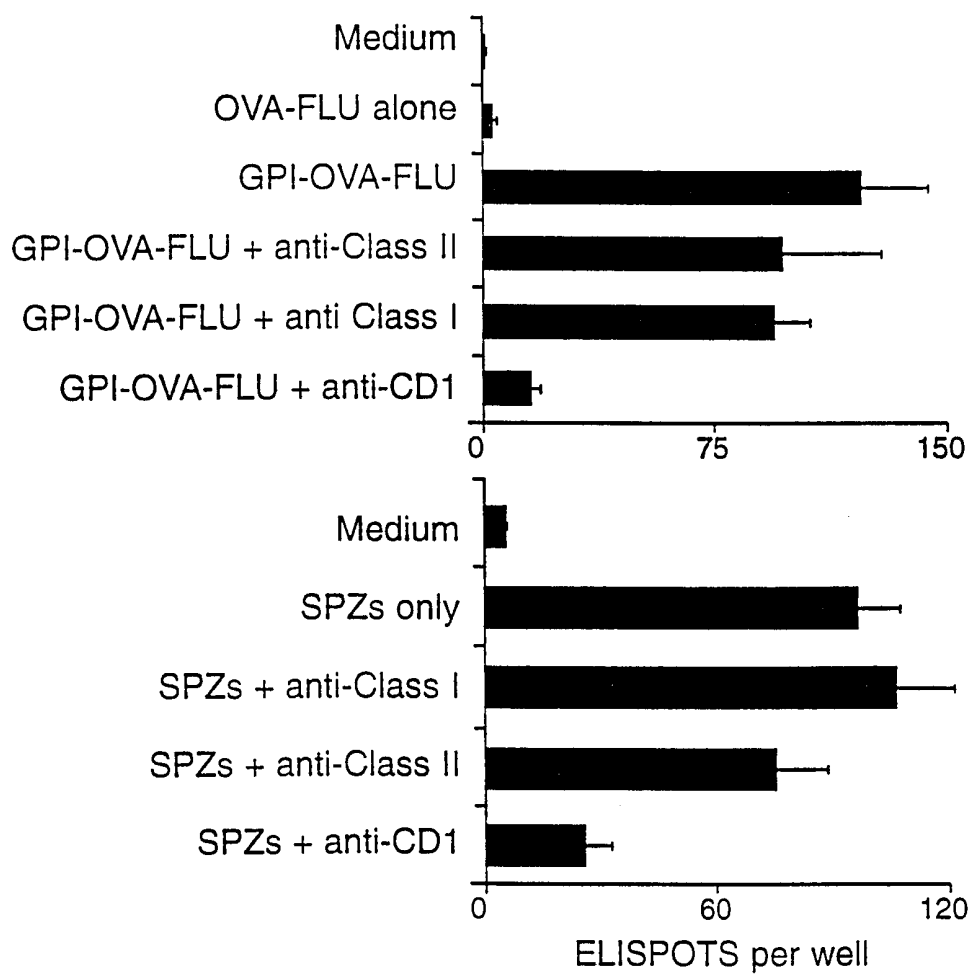
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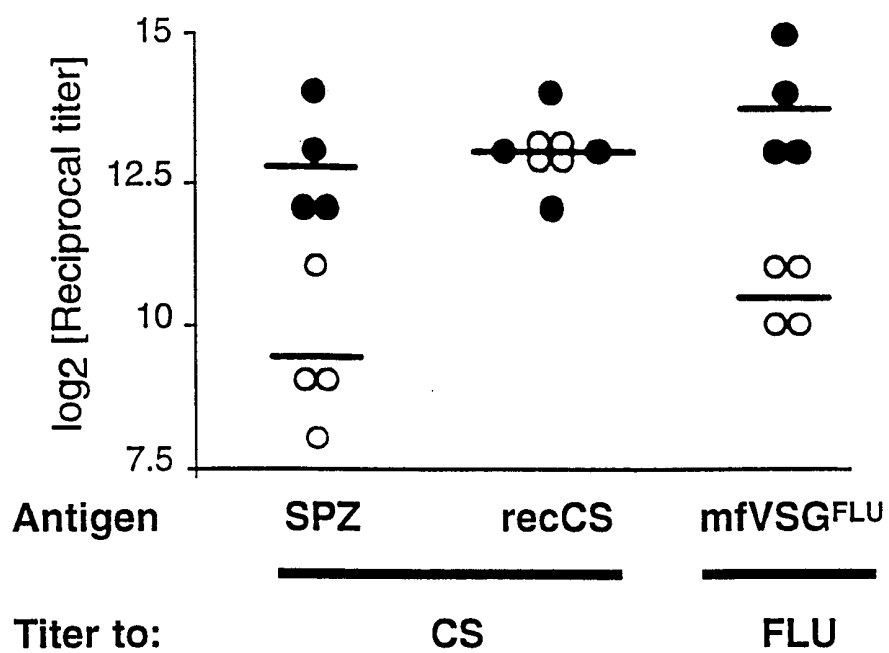
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00929

A. CLASSIFICATION OF SUBJECT MATTER					
Int Cl ⁶ : A61K 31/72, 31/73, 39/015, 39/008, 121:00					
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) A61K					
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) WPAT; CAPLUS; MEDLINE: CDI, Glycosylphosphatidylinositol and T-Cell					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
X,Y	WO 99/52547 (BRIGHAM WOMEN'S HOSPITAL INC.) EPD 13 April 1993 A61K 39/04, 39/39 OPI 21 October 1999. Page 3, lines 9-21 and Claims 14, 29 and 30	1-72			
X,Y	Nagata, Norikazu; Taketani, Shigeru; Adachi, Yasushi; Hosaka, Naoki; Miyashima, Shigeo; Tokunga, Rikio; Ikehara, Susumu. A monoclonal antibody reactive with a glycosylphosphatidyl inositol-anchored molecules on T-cells defines CD4+ T cells subsets. Eur J Immunology. Volume 25, No: 3 11193-6. 1993.	1-72			
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex					
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> * 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 </td> <td style="width: 33%; vertical-align: top;"> "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 </td> <td style="width: 33%;"></td> </tr> </table>			* 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	
* 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 27 November 1999		Date of mailing of the international search report 23 DEC 1999			
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No.: (02) 6285 3929		Authorized officer A. WILCOX Telephone No.: (02) 6283 2243			

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00929

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	Schofield, Louis; McConville, Malcolm, J; Hansen, Diana; Campbell, A Stewart; Fraser-Reid, Bert; Grusby, Michael, J; Tachado, Souvenir D. CDId-restricted immunoglobulin G formation to GPI anchored antigens mediated by NKT cells. Science 1983, Volume 283, No: 5399:225-229.	1-72
X,Y	Joyce, Sebastian; Woods, Amina S; Yewdell, Jonathon W; Bennick, Jack R; DeSilva, A Darshan; Boesteanu, Alina; Balk Steven P; Cotter, Robert J; Brutkiewicz, Randy R. Natural Ligand of mouse CDId:cellular glycosylphosphatidylinositol. Science 1998 Volume 279, No: 5356:1541-1544.	1-72
X,Y	Masuda, T; Yonemura, Y; Fujimoto, K; Hidaka, M; Nagakura, S; Nakakuma, H; Hata, H; Sanada, I; Kawakita, M and Takatsuki, K. Establishment of a human T-cell line with deficient surface expression of glycosylphosphatidylinositol (GPI)-anchored proteins from a patient with paroxysmal nocturnal haemoglobinuria. British Journal of Haematology, 1994, Volume 87, No: 1:24-30.	1-72
X,Y	Del-Porto, P; Mami-Chouaaib, F; Bruneau, J M; Jitsukawa, S; Dumas, J; Harnois, M and Hercend, T. TCT.1, a target molecule for gamma delta T cells is encoded by an immunoglobulin superfamily gene (Blast-1) located in the CD-1 region of human chromosome 1. Journal of Experimental Medicine. 1991, Volume 173, No: 6:1339-44.	1-72
X,Y	Beckman, Evan M; Porcelli, Steven A; Morita, Craig T; Behar, Samuel A; Furlong, Stephen T and Brenner, Michael B. Recognition of a lipid antigen by CDI-restricted $\alpha\beta^+$ T cells. Nature. Volume 372:691-694, 1994.	1-72
X,Y	Sieling, P. A; Chatterjee, D.; Porcelli, S A.; Prigozy, TI; Mazzaccaro, R J; Soriano, T; Bloom, B R; Brenner, M B; Kronenberg, M; Brennan, P J and Modlin, R L. CD-1-Restricted T Cell Recognition of Microbial Lipoglycan Antigens. Science. Volume 269:227-229, 1995.	1-72

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 99/00929

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The feature common to all of the claims is a glycosylphosphatidylinositol (GPI) which is capable of interacting with a CDI on an immune cell to form an association with CDI which association activates helper T cells. However, this common feature is generic in the art. Consequently, the common feature does not constitute "a special technical feature" since it makes no contribution over the prior art. Since there exists no other common feature which can be

continued on extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

international application No.

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Box II (continued)

considered as a special technical feature, no technical relationship between the different inventions can be seen. Consequently, it appears that a posteriori, the claims do not satisfy the requirement that they relate to one invention only.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00929

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	99/52547	NONE
END OF ANNEX		