Title: NATURALLY PROCESSED IMMUNODOMINANT PEPTIDES DERIVED FROM NEISSERIA MENINGITIDIS PORIN A PROTEIN AND THEIR USE

Abstract: The present invention relates to precisely identified naturally processed immunodominant peptides derived from N. meningitidis serotype B, that may be used in ex vivo diagnosis of human MHC class II restricted immune responses to meningococcal antigens. The naturally presented immunodominant peptides are highly immunogenic in vivo and are capable of recalling an antigen specific CD4+ T cell response ex vivo. The immunodominant PorA-derived peptides may further be used in methods for vaccination against N. meningitidis serotype B.
Naturally processed immunodominant peptides derived from *Neisseria meningitidis*

Porin A protein and their use

Field of the invention

The present invention relates to naturally processed immunodominant peptides derived from *Neisseria meningitidis* and methods of using these peptides in *ex vivo* diagnosis of human MHC class II restricted immune responses to *N. meningitidis*, in particular to serogroup B. The immunodominant peptides are preferably derived from the Porin A (PorA) protein of *N. meningitidis*. The invention further relates to vaccines against *N. meningitidis* comprising at least one antigenic peptide of the invention.

Background of the invention

Infections by *Neisseria meningitidis* serogroup B (Men B) are the most common cause of bacterial meningitis or sepsis in the western world (Rosenstein et al. 2001, *N. Engl. J. Med.* 344, 1378-1388). The natural habitat of the meningococcus is the nasopharynx, and meningococcal carriage, a naturally immunizing status, occurs in approximately 10% of the population. The development of meningococcal disease depends on poorly defined environmental, microbial and (immuno)genetic factors. The rapid increase in the incidence of meningococcal disease, with high morbidity and mortality in previously healthy young infants and adolescents, urges the need for a vaccine (Jodar, L. et al. 2002, *Lancer* 359, 1499-1508).

The polysaccharide of the Men B serogroup is poorly immunogenic and alternative vaccine candidates, including outer membrane proteins (OMP), have been investigated. For example WO90/06696 describes the isolation of class 1 OMP’s and suggest their use in immunization against meningococcal disease. However, the OMP class 1 peptides identified therein lack explicit HLA specificity.

High affinity, bactericidal antibody titers against the variable major outer membrane protein PorA are thought to protect against invasion of the meningococcus and disease. However, preclinical studies using candidate Men B vaccines based on
outer membrane vesicles (OMV) from genetically manipulated meningococci point out that different PorA wildtypes may not be equally immunogenic (Rouppe et al. 2000 Vaccine 18, 1334-1343; de Kleijn et al. 2001, Vaccine 20, 352-358; de Kleijn et al. 2000, Vaccine 18, 1456-1466). Since the induction and maintenance of anti-PorA antibody titers are typically T cell dependent, dissection of the MHC class II (Major Histocompatibility Complex II) restricted CD4+ T helper cell response against this protein is paramount for further vaccine improvement. While meningococcal OMP, including PorA, have been shown to be immunogenic to human CD4+ T cells (Oftung et al. 1999 Infect. Immun. 67, 921-927; Wiertz et al. 1996, Infect. Immun. 64, 298-304), only a few PorA specific T cell epitopes were identified by classical epitope mapping using synthetic peptides in T cell assays (Wiertz et al. 1992, J. Exp. Med. 176, 79-88).

T cells recognise naturally processed peptides presented by MHC molecules at the cell surface of professional antigen presenting cells (APC) (Watts 2001, Curr. Opin. Immunol. 13, 26-31; Yewdell & Bennink 2001 Curr. Opin. Immunol. 13, 13-18), and T cell epitopes from defined antigens may be predicted using MHC peptide binding algorithms (Rotzschke et al. 1991, Eur. J. Immunol. 21, 2891-2894; Manici et al. 1999, J. Exp. Med. 189, 871-876). However, particularly for CD4+ T cell epitopes this approach has a high failure rate because the motifs for binding to MHC class II molecules are little confined (Chicz et al. 1993, J. Exp. Med. 178, 27-47; Rammensee et al. 1995, Immunogenetics 41, 178-228), and, moreover, because predicted epitopes may not be naturally processed in the cell. Furthermore, the exact composition of a naturally processed epitope, especially the set of N-terminal and C-terminal residues flanking the MHC class II binding core region, cannot be predicted. Yet, these are of great importance for the outcome of the immune response (Moudgil et al. 1998, Imm. Today, 19, 217-220).

In view of the above, there remains a need for identification of PorA derived, immunogenic peptides, most desirably in their naturally occurring length, for use in diagnostic, prophylactic and therapeutic methods in T cell dependent protective immunity against meningococcal serogroup B infection. The present invention made use of mass tag assisted chemical analytical identification to map naturally processed
and HLA-class II presented peptides derived from PorA, which are recognized by CD4+ T cells.

**Description of the invention**

The present invention is based on the chemical analytical identification of MHC class II haplotype specific, naturally processed and presented PorA-derived immunodominant peptides, which are recognized by CD4+ T cells. For example, at least six PorA amino acid regions are described, in which the exact naturally processed and HLA-DR-presented PorA epitopes were identified, including peptides presented by two different MHC-II alleles, for example by HLA-DRB1*0101 and HLA-DRB1*1501. Also included is one other novel immunodominant PorA region (region 3) identified by classical epitope mapping using synthetic peptides and T cell assays.

The present invention relates to naturally processed PorA-derived antigenic peptides and their use in *ex vivo* diagnosis of human immune responses and in vaccine development. The terms “naturally processed PorA-derived antigenic peptides” or “naturally processed PorA-derived immunodominant peptides” as used herein refer to various antigenic peptides comprised within PorA-derived antigenic regions of the *N. meningitidis* PorA protein, such as, but not limited to, the peptides selected from Table 1, or fragments or variants thereof. The term “naturally processed PorA-derived antigenic region(s)” in Table 1 refers for example to region 1 (from about amino acid 1 to about amino acid 20; SEQ ID NO: 1; SEQ ID NO:2) of the PorA protein of *N. meningitidis* serogroup B (amino acid numbering is based on the P1.5-2,10 serosubtype after cleavage of the N-terminal signal peptide, but the same regions and amino acid sequences also occur with slightly different numbering in other PorA serosubtypes), and likewise this term refers to the other regions indicated in Table 1. Also comprised within the definition of naturally processed PorA-derived antigenic regions are the nucleic acid sequences of the *porA* gene encoding these regions. Due to the degeneracy of the genetic code, various nucleic acid sequences may encode the amino acid sequences of the regions of Table 1. Likewise, strain variants (as defined below) of the amino acids of peptides selected from Table 1 are included in this definition.
Naturally processed PorA-derived antigenic peptides are for example the peptides selected from Table 1, or fragments, such as a minimal core region, or strain variants of the peptides or peptide fragments. A "minimal core region" is a fragment of an PorA-derived antigenic peptide, which is still antigenic and retains its ability to be recognized by CD4+ T cells and induce an immune response in subjects. The minimal core region of an antigenic peptide according to the invention can easily be determined by for example terminal amino acid deletions followed by functional assays, as described in the Examples. For example, the minimal core region of peptide PorA1.5-2,1091-106 was determined to comprise amino acids 94 to 104 (EFGLRAGRVA; amino acids 4-14 of SEQ ID NO: 2), with F95 (amino acid 5 of SEQ ID NO: 5) likely serving as the P1 peptide anchor residue for HLA-DRB1*0101 binding.

The term "variants", when used in connection with peptides or nucleic acid sequences, refers to naturally processed PorA-derived antigenic peptides or peptide fragments or nucleic acid sequences encoding these, whereby the peptides are still antigenic and retain their ability to be recognized by CD4+ T cells and induce an immune response in subjects. For example, single amino acid substitutions or deletions, or length alterations of the peptides selected from Table 1 may be carried out without diminishing the antigenic properties of the peptides. The invention provides for example naturally occurring length variants of each of the PorA-derived antigenic regions 1, 4 and 7. Region 1 comprises for example the length variants P1.5-2,101-18 (SEQ ID NO: 1) and P1.5-2,101-20 (SEQ ID NO: 2). All three regions 1, 4 and 7 are membrane spanning regions, generally conserved among different serotypes of meningococcal PorA proteins. The peptide P1.5-2,10349-367 (amino acids 1-16 of SEQ ID NO: 13 followed by amino acids 13-15 of SEQ ID NO: 15), comprising region 7, is also one embodiment of the invention.

Variants can be easily generated and tested for the retention of functionality using methods known in the art, such as but not limited to amino acid substitutions or deletions, de novo chemical synthesis of peptides or mutagenesis- or gene-shuffling techniques, hybridization techniques to isolate variants from N. meningitidis strains, and the like. Variants of naturally processed PorA-derived antigenic peptides as defined herein include peptides with amino acid sequences with at least 80%, 90%, 95% or 99%
"substantial sequence identity" (as defined below) to the naturally processed PorA-derived antigenic peptides selected from Table 1, which retain their ability to be recognized by CD4+ T cells and induce an immune response in subjects.

It is noted, that, although the peptides of the invention were identified in the *N. meningitidis* serogroup B PorA protein P1.5-2,10, they may also occur in other serogroup B serosubtypes of PorA, or in PorA proteins of other *N. meningitidis* serogroups, such as for example serogroups A or C, and can thus also be isolated from strains with these PorA proteins.

In one embodiment of the invention the naturally processed PorA-derived antigenic peptides are HLA class II allele-specific, preferably HLA-DR specific. "HLA-DR specific" as used herein refers to the preferential (dominant) presentation of an antigenic peptide by a particular HLA-DR molecules, in accordance with Table 1. HLA-DR typing and HLA-DR subgroup typing can be done using various methods known in the art, such as marker analysis (RFLP, AFLP, oligonucleotide typing, etc.) or serological methods. HLA-DR alleles may be subdivided into subgroups such as DRB1*0101, DRB1*0102 and DRB1*0103.

In another embodiment of the invention the antigenic peptides are dominantly presented in the context of at least two HLA-DR alleles, such as alleles selected from e.g. HLA-DR1, HLA-DR2, HLA-DR3, HLA-DR4, HLA-DR7, HLA-DR8, HLA-DR9, HLA-DR10, HLA-DR11, HLA-DR12, HLA-DR13, HLA-DR14, or HLA-DR15. For example the peptides of SEQ ID NO: 1 and 2 (P1.5-2,10,1-18 and 1-20) are presented by HLA-DRB1*0101 and HLA-DRB1*1501, and can thus be used in diagnosis or treatment of subjects comprising at least the HLA class II alleles HLA-DR1 and/or HLA-DR15. A peptide which is presented by two HLA alleles is also referred to as being "allele specific" herein, as it is preferentially (dominantly) presented by two specific HLA-DR alleles. It is then referred to as "HLA-DRX and HLA-DRY specific" peptide, wherein DRX and DRY are two different HLA class II alleles selected from the above.

One aspect of the invention relates to methods for *ex vivo* diagnosis of MHC class II restricted immune responses to Men B antigens in a subject using the identified naturally processed PorA derived antigenic peptides. The method comprises the steps
of: (a) determining the MHC class II haplotype of the subject, for example determining whether the subject is HLA-DR1 and/or HLA-DR15 positive; (b) providing a composition comprising peripheral blood mononuclear cells (PBMC's) from the subject; (c) mixing the composition comprising PBMC's with at least one soluble peptide that comprises a naturally processed PorA-derived antigenic peptide sequence selected from Table 1 or a fragment, variant, or HLA-peptide multimers derived from the antigenic peptide sequence, whereby the antigenic peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1 or whereby the peptide matches a cross-presenting variant (allele) thereof, and, (d) determining the presence of MHC class II restricted immune cells, specific for the peptide(s), in the subject's PBMC's. In a preferred embodiment the soluble peptide comprises a peptide sequence selected from Table 1. In a further preferred embodiment the soluble peptide comprises only a peptide sequence selected from Table 1, i.e. consists of a peptide sequence selected from Table 1. Alternatively, the soluble peptide may comprise one or more peptide sequences selected from Table 1 linked to or flanked by further amino acid sequence(s), whereby preferably at least one further amino acid sequence is different from the amino acid sequence with which the peptide is natively associated in a naturally occurring porin A protein of *N. meningitides*.

A "cross-presenting" or "functionally matching" haplotype herein refers to the phenomenon wherein a single peptide epitope is presented by more than one HLA allele of which the binding grooves are structurally related. Examples of "cross-presenting" or functionally matching haplotypes are e.g. HLA-DRB1*0101 and HLA-DRB1*0102, or HLA-A*0201 and HLA-A*0205 (Lund, O. et al. 2004, Immunogenetics 55, 797-810). Two different PBMC populations expressing different but "cross-presenting" or "functionally matching" haplotypes will excite a similar response from a T cell population when challenged with one and the same peptide epitope, whereby the response preferably does not differ by more than a factor 10, 5, 2, or 1.5.

The MHC class II haplotype refers to the set of linked HLA class II alleles found on the same chromosome. In step (a) of the method, the MHC class II haplotype of the subject, and in particular whether the subject is positive for certain HLA-DR alleles (such as but not limited to HLA-DR1 and/or HLA-DR15 and/or other alleles), is
determined using any one of a number of methods well known in the art, (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). Whether a subject is homozygous or heterozygous for a particular HLA class II allele can also be determined. In step (b) of the method, a composition comprising PBMC's from the HLA-DR selected or typed (human) subject is obtained using a variety of methods well known in the art (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., 711-712). Blood samples are usually processed to remove erythrocytes and platelets (e.g., by aphaeresis, Ficoll density gradient centrifugation and/or red blood cell lysis or other such methods known to one of skill in the art) and the remaining PBMC sample, which includes the T-cells of interest, as well as B-cells, macrophages and dendritic cells, may be used directly in the assay. The composition comprising PBMC's may e.g. consist of the PBMC bulk that is obtainable from blood donated by the subject. For higher yields of PBMC’s, the subject from whom the PBMC’s are obtained may be given a pre-treatment with GM-CSF for mobilising mononuclear cell subpopulations. The PBMC composition may further be enriched for specific subsets of mononuclear cells, preferably T cells, more preferably CD4+ T cells. The PBMC composition may be enriched for T cells, or in particular for CD4+ T cells, by methods known in the art, such e.g. by expansion of T cells or CD4+ T cells as described e.g. in Coligan et al. (1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., 1994: 711-94).

Subsequent to having established whether a subject is positive for at least one of the HLA class II restricting alleles, and to having prepared a PBMC sample, in step (c) of the diagnostic methods of the invention the PBMC's (of known HLA class II haplotype) are mixed with a composition comprising at least one soluble peptide that comprises the minimal core region of a PorA-derived antigenic peptide sequence selected from Table 1 or a fragment, variant, or HLA-peptide multimer derived from the antigenic peptide sequence, whereby the antigenic peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1. In a specific embodiment, the peptide of step (c) is a PorA derived protein sequence including at least the minimal core region of a PorA-derived antigenic peptide sequence depicted in Table 1,
preferably the PorA derived protein sequence includes at least one of the peptide sequences depicted in Table 1, or variants thereof. Variants may be longer or shorter length variants or strain variants.

The naturally processed PorA-derived peptides of the invention may be obtained as described below. The soluble peptide(s) to be mixed with the composition comprising the PBMC's of a given subject selected to express the restricting HLA class II allele(s) preferably comprise(s) an amino acid sequence of at least 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, or 32 contiguous amino acids of peptides selected from Table 1. Preferably soluble peptides are brought into contact with the composition comprising the PBMC's at a concentration that ranges from 1 nM to 50 μM, more preferably 10 nM to 20 μM, most preferably 100 nM to 10 μM. The amount of PBMC's to be brought into contact with the peptide depends on the assay format used for detecting the PBMC response and is well known in the art. Various such assays are described below and the amount of PBMC's to be used in these assays are known to the skilled person. Preferably only those peptides that match the MHC class II haplotype of the subject in accordance with Table 1 are mixed with the composition comprising the subject's PBMC's.

The term "HLA-peptide multimer" of PorA refers to complexes comprising several purified and multimerized HLA-molecules (e.g. tetramers) each binding the same PorA derived peptide, or fragment or variant thereof, as described in Altman et al. (Science 1996, 274(5284): 94-6), incorporated herein by reference. For example, an HLA-peptide multimer may comprise purified HLA-DR multimers to which one of the PorA derived peptides of the invention is bound and which further comprises a fluorescent label.

multimers are brought into contact with the composition comprising the PBMC’s at a concentration of 2-20 μg/ml. All methods include recombinant protein technology and (bio)chemistry to synthesize, purify, refold, multimerise and fluoresceine-label HLA molecules for use in flow cytometry.

Finally, in step (d) of the method, the presence of human MHC class II restricted immune cells in PBMC’s specific for the PorA-derived antigenic peptide(s) is determined. The response of the PBMC’s may be determined using a number of methods available in the art (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). One method to determine the antigen specific response of the PBMC’s is by measuring the proliferative capacity of the PBMC’s in the presence of the soluble PorA-derived antigenic peptide(s). More preferably, the proliferation of T cells in response to the PorA-derived antigenic peptide(s) is determined. The proliferation of T cells in response to the soluble PorA-derived antigenic peptide(s) is preferably determined directly in the composition comprising the PBMC’s, i.e. without pre-expansion of the T cells. Alternatively, the T cells or more specifically, the CD4⁺ T cells may be enriched or pre-expanded to increase the sensitivity of the diagnostic method of the invention. In a further preferred method, the proliferation of CD4⁺ T cells (in the composition comprising PBMC’s) in response to the presence of the soluble PorA-derived antigenic peptide(s) is determined.

Alternatively, the presence of effector cell function of the CD4⁺ T cells in response to the presence of the soluble PorA-derived antigenic peptide(s) may be determined using a variety of methods available in the art, (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). In a preferred method the effector cell function of the PBMC’s, in particular the CD4⁺ T cells is determined by measuring the production or secretion of a "soluble protein factor" by PBMC’s or T-cells in response to soluble peptide stimulation. A variety of soluble protein factors can be detected by the assays disclosed herein. The soluble factors may be cytokines, lymphokines or chemokines. Typically this secreted factor is a lymphokine, such as enumerated below. As a result of the increased sensitivity of the assay, factors secreted by rare T-cells
which occur in low frequency can be detected. Factors which are detected by this method include, but are not limited to lymphokines, cytokines and chemokines such as for example, IFN-γ, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-10, IL-13, TGF-β, RANTES, and GM-CSF.

A variety of assay formats can be used to detect the increased secretion levels of factors produced by the composition described herein. Suitable assays include both solid phase and non-solid phase protocols. The assays can be run using competitive or non-competitive formats, and using a wide variety of labels, such as radioisotopes, enzymes, fluoroscers, chemiluminescers, spin labels, and the like. Such methods include, but are not limited to enzyme-linked immunosorbent assays (ELISA), both direct and reverse formats, and other assays such as LUMINEX, cytokine secretion (capture-) and ELISPOT tests. It will be recognised that negative controls, i.e., samples run without added antigen, and positive controls, i.e., samples run with antigens, such as tetanus toxoid, known to elicit lymphokine secretion from T-cells will be run as necessary under otherwise duplicative conditions to validate the assay results.

Some assays rely on solid phase protocols where a ligand complementary to the secreted factor (such as antibody against the secreted factor) is bound to a solid phase which is used to capture the secreted factor. The ligand may be conveniently immobilised on a variety of solid phases, such as dipsticks, particulates, microspheres, magnetic particles, test tubes, microtiter wells, and plastics, nitrocellulose or nylon membranes and the like, including polyvinyl difluoride (PVDF) (e.g., 96 well plate with a PVDF membrane base (Millipore MAIPS45-10)) and ELISA grade plastic. The captured factor can then be detected using the non-competitive "sandwich" technique where a directly or indirectly labelled second ligand for the factor is exposed to the washed solid phase. Such assay techniques are well known and well described in both the patent and scientific literature. See, e.g., U.S. Pat. Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Enzyme-linked immunosorbent assay (ELISA) methods are described in detail in U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; and 4,034,074. ELISA assays detect very

A commonly used assay format to measure cytokine secretion is the antibody capture assay. The general protocol is simple: a ligand, e.g., an unlabeled antibody for the secreted factor, is immobilized on a solid phase, and the secreted factor is allowed to bind to the immobilized antibody. The bound secreted factor is then detected by using a labelled secondary reagent that will specifically bind to the captured factor ("direct sandwich assay"). Alternatively, the secondary reagent will not be labelled, but will be detected by subsequent binding to labelled tertiary binding reagent complementary to the second binding reagent ("indirect sandwich assay"). The strength of signal from the bound label allows the determination of the amount of secreted factor present in the sample and this in turn allows the quantification of the number of activated T-cells present in the sample. As will be recognised by the skilled person, the sandwich assay can be used to detect any secreted factor which has two epitopes, each of which can be recognised by the specific binding pair members. Choosing appropriate capture and detection antibody pairs permits the application of this assay to the detection of T cells secreting a variety of soluble factors. A list of antibody pairs which can be used in this assay is inter alia presented in U.S. Pat. No. 6,218,132.

Alternatively, the presence of effector cell function of the T cells may be determined by measuring the production of cytokine by intracellular cytokine staining as e.g. described by Murali-Krishna et al. (1998, Immunity 8: 177-187).

A preferred cytokine for determining the effector cell function of the CD4+ T cells in response to the presence of the naturally processed PorA-derived peptide(s) is IFN-γ. Again, methods for measuring antigen induced IFN-γ production by the CD4+ T cells are well known in the art (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 6: Cytokines), and include e.g. a method wherein IFN-γ production is measured in an elispot assay, as described in the Examples herein. Preferably, the IFN-γ production is measured in a direct ELISPOT or intracellular cytokine assay, whereby "direct" is understood to mean directly on the composition of PBMC's obtained from the subject, without pre-expansion of the T cells or CD4+ T cells. Likewise, the other above-mentioned
lymphokines and cytokines may be measured in a direct ELISPOT or intracellular cytokine assay.

Finally, another preferred method for determining the presence of human MHC class II restricted immune cells in PBMC's specific for the naturally processed PorA-derived antigenic peptide(s) is by *ex vivo* enumeration of specific HLA-peptide multimer binding T cells in PBMC samples using flowcytometry. Only T cells specific for a particular peptide presented by a particular MHC class II allele will bind fluorescent HLA-peptide multimers made of these components. Again, methods for measuring HLA-peptide multimer binding are well known in the art and examples thereof can be found in literature (see e.g. Altman J.D. et al. Science 1996, vol 274, 94-96; Cameron T.O. et al, Journal of Immunol. Methods 2002, vol 268, 51-69; Kwok, W.O. et al, Journal of Immunol. Methods 2002, vol 268, 71-81; Hugues S. et al, Journal of Immunol. Methods 2002, vol 268, 83-92, all three incorporated herein by reference) After mixing a subject's PBMC's with fluorochrome labeled HLA-peptide multimers prepared using matching HLA-DR molecules and PorA derived antigenic peptides, in accordance with Table 1, and with various fluorescent reagents specific for T cell markers, MHC class II restricted immune T cells in PBMC's specific for the naturally processed PorA-derived antigenic peptide(s) can be quantitated in PBMC's by counting cells simultaneously binding various fluorochromes in a tri-or four-colour FACs analysis.

In yet another embodiment, the invention relates to diagnostic kits for *ex vivo* diagnosis. Such kits comprise for example one or more of the antigenic peptides of the invention and/or fluorescent HLA-DR-peptide multimers hereof (such as dimeric or tetrameric complexes, liposomes and the like) reagents, which are recognized and/or bind to MHC class II restricted immune cells specific for the PorA derived peptides. In addition the kits may comprise one or more detection components, depending on the detection assay to be used, and instructions for use.

The diagnostic methods of the invention may be used for a variety of reasons on different subjects. The methods may e.g. be used on a subject (infant, child or adult) showing symptoms of meningococcal infection, such as stiff neck, high fever,
sensitivity to light, confusion, headaches and vomiting or in certain extreme cases haemorrhagic rash and rapid circulatory collapse, in order to diagnose whether the disease symptoms are caused by Men B and to distinguish between infections involving similar symptoms. Also, infection may be diagnosed in carriers, where no disease symptoms are apparent. Having established the diagnosis of a Men B infection in a given subject, the diagnostic methods of the invention may further be used to monitor the immune response to antigens in the subject undergoing an Men B infection. The diagnostic methods may further be used to determine the status of a subject's immune response in a subject having undergone an infection with Men B, i.e. in a subject that no longer shows any clinical symptoms of Men B infection. Finally, the methods of the invention may be used to determine the status of a subject's immune response in a subject having been vaccinated against Men B. In particular, the methods may be used to evaluate correlates of protection in individuals having been vaccinated against Men B.

In a further aspect, the invention relates to a method for vaccination or immunisation of a subject against Men B, preferably against several strains of Men B. The method comprises administering to the subject a pharmaceutical composition comprising a protein or peptide comprising at least one naturally processed PorA-derived antigenic peptide, selected from Table 1, or a fragment or variant thereof. In a preferred method, the peptide that is administered matches the MHC class II haplotype of the subject. Such preferred methods may thus include the further step of: determining the MHC class II haplotype of the subject, in particular determining whether the subject is positive for at least one specific HLA-DR allele. Thus in the preferred method, a subject that is determined positive for a certain HLA-DR allele, may be administered a pharmaceutical composition comprising a peptide or protein comprising a naturally processed PorA-derived antigenic peptide sequence selected from Table 1, or a variant or fragment of the peptide sequence, whereby the peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1 or whereby the peptide matches a cross-presenting variant (allele) thereof. In these methods, a fragment comprises preferably at least 9, 10, 11, 12 or 13 contiguous amino acids of peptide
sequences depicted from Table 1, or variants thereof. In these methods, the presence of the HLA-DR allele(s) of the subject is determined by known methods as described above. Method for preparing the peptides to be used in this method, as well as pharmaceutical composition comprising these peptides and methods for their preparation are described herein below. In a preferred method of vaccination, a pharmaceutical composition suitable for parenteral administration is administered parenterally, a pharmaceutical composition suitable for transdermal administration is administered transdermally, or a pharmaceutical composition suitable for inhalation is inhaled.

In yet another aspect, the invention relates to the use of a naturally processed PorA-derived antigenic peptide as defined above for the manufacture of a vaccine for prophylaxis and/or therapy of Men B infection in a subject. The PorA-derived peptide preferably matches the MHC class II haplotype of the subject, i.e. for a subject positive for the HLA-DRB1*0101 allele for example SEQ ID NO’s 1,2,5-8, 10, 13,14 and 16 or fragments or variants thereof may be used, and for subjects positive for the HLA-DRB1*1501 allele SEQ ID NO 1-3, 5,9, 11, 12, 15 and 16 or fragments or variants thereof may be used. Preferably, the vaccine is a pharmaceutical composition suitable for parenteral or transdermal administration.

It is also envisaged that the DNA encoding one or more naturally processed PorA-derived antigenic peptides is used as a DNA-vaccine, as for example described in US 6,586,409 or in WO03/048371, incorporated herein by reference. In DNA vaccination DNA encoding an antigenic peptide is introduced into human or animal cells by various methods, and expression of the peptide leads to a protective immune response. Preferably, again the antigenic peptide encoded by the DNA, matches the MHC class II haplotype of the human or animal subject into which the DNA is introduced.

Peptides for use in the methods of the invention

The peptides of the invention contain an epitope (or antigenic determinant) that are specifically presented by MHC class II molecules, i.e. each peptide derived from a PorA-derived antigenic region of the invention is specifically presented by a particular HLA-DR molecule, as described in Table 1, whereby certain peptides, such as for
example SEQ ID NO 1 and fragments or variants thereof of PorA-derived antigenic region 1, are specifically presented by two HLA-DR molecules (i.e. HLA-DRB1*0101 and/or HLA-DRB1*1501). The peptides of the invention thus bind the groove or cleft of the MHC class II molecule (or molecules) in question. The peptides of the invention typically comprise at least about 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 amino acid residues. In certain embodiments the peptides will be larger but will typically not exceed about 32 residues. Thus, a wide range of peptide sizes may be used in the present invention.

Particularly because the peptides to be used in the methods of the invention may be relatively short, the peptides can be readily synthesised using known methods. For example, the peptides can be synthesised by the well-known Merrifield solid-phase synthesis method in which amino acids are sequentially added to a growing chain. See Merrifield (1963), *J. Am. Chem. Soc.* 85:2149-2156; and Atherton et al., “Solid Phase Peptide Synthesis,” IRL Press, London, (1989). Automatic peptide synthesisers are commercially available from numerous suppliers, such as Applied Biosystems, Foster City, California. Additional synthetic approaches for preparing the peptides of the invention are described in the Examples herein.

Alternatively, the naturally processed PorA-derived peptides may be prepared as part of larger polypeptides comprising one or more of the peptides of the invention. Such larger polypeptides may be prepared using well-known recombinant techniques in which a nucleotide sequence encoding the polypeptide of interest is expressed in cultured cells such as described in Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York (1987) and in Sambrook and Russell (2001) "Molecular Cloning: A Laboratory Manual (3rd edition), Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, New York, both of which are incorporated herein by reference in their entirety. Also see, Kunkel, 1985, *Proc. Natl. Acad. Sci.* 82:488 (describing site directed mutagenesis) and Roberts *et al.*, 1987, *Nature* 328:731-734 or Wells, J.A., et al. (1985) Gene 34:315 (describing cassette mutagenesis). For example, a *porA* gene of *N. meningitidis* serogroup B may be cloned and expressed in its entirety and the antigenic peptide may be derived from the entire protein or the desired fragments (encoding the desired antigenic region(s) or peptide(s))
may be cloned and expressed individually. Isolated nucleic acid sequences encoding
PorA are widely available to the skilled person, as is porA nucleic acid and PorA amino
acid sequence information from sequence databases such as SwissProt, EMBL,
GenBank, etc.

Typically, nucleic acids encoding the desired polypeptides are used in expression
vectors. The phrase "expression vector" generally refers to nucleotide sequences that are
capable of affecting expression of a gene in hosts compatible with such sequences.
These expression vectors typically include at least suitable promoter sequences and
optionally, transcription termination signals. Additional factors necessary or helpful in
effecting expression may also be used as described herein. DNA encoding the
polypeptides of the present invention will typically be incorporated into DNA constructs
capable of introduction into and expression in an in vitro cell culture. Specifically, DNA
constructs will be suitable for replication in a prokaryotic host, such as bacteria, e.g.,
E. coli, or may be introduced into a cultured mammalian, plant, insect, yeast, fungi or
other eukaryotic cell lines. Transgenic plants expressing the PorA-derived peptides of
the invention, as for example described in US2002090371 or US6338850, may also be
generated. The derived plant tissue comprising the peptides may be used directly for
oral administration or processed further.

DNA constructs prepared for introduction into a particular host will typically
include a replication system recognised by the host, the intended DNA segment
encoding the desired polypeptide, and transcriptional and translational initiation and
termination regulatory sequences operably linked to the polypeptide encoding segment.
A DNA segment is “operably linked” when it is placed into a functional relationship
with another DNA segment. For example, a promoter or enhancer is operably linked to
a coding sequence if it stimulates the transcription of the sequence. DNA for a signal
sequence is operably linked to DNA encoding a polypeptide if it is expressed as a
preprotein that participates in the secretion of the polypeptide. Generally, DNA
sequences that are operably linked are contiguous, and, in the case of a signal sequence,
both contiguous and in reading frame. However, enhancers need not be contiguous with
the coding sequences whose transcription they control. Linking is accomplished by
ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof.
The selection of an appropriate promoter sequence generally depends upon the host cell selected for the expression of the DNA segment. Examples of suitable promoter sequences include prokaryotic, and eukaryotic promoters well-known in the art. See, e.g., Sambrook and Russell (2001, *supra*). The transcriptional regulatory sequences will typically include a heterologous enhancer or promoter which is recognised by the host. The selection of an appropriate promoter will depend upon the host, but promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters are known and available. See, e.g., Sambrook and Russell (2001, *supra*).

Conveniently available expression vectors which include the replication system and transcriptional and translational regulatory sequences together with the insertion site for the polypeptide encoding segment may be employed. Examples of workable combinations of cell lines and expression vectors are described in Sambrook and Russell (2001, *supra*). For example, suitable expression vectors may be expressed in, e.g., insect cells, e.g., Sf9 cells, mammalian cells, e.g., CHO cells and bacterial cells, e.g., *E. coli*.

It will be understood that the PorA-derived (poly)peptides of the invention may be modified to provide a variety of desired attributes, e.g., improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide. For instance, the peptides can be modified by extending, decreasing the amino acid sequence of the peptide. Substitutions with different amino acids or amino acid mimetics can also be made.

The individual residues of the immunogenic PorA-protein derived (poly)peptides of the invention can be incorporated in the peptide by a peptide bond or peptide bond mimic. A peptide bond mimic of the invention includes peptide backbone modifications well known to those skilled in the art. Such modifications include modifications of the amide nitrogen, the α-carbon, amide carbonyl, complete replacement of the amide bond, extensions, deletions or backbone cross-links. See, generally, Spatola, *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. VII (Weinstein ed., 1983). Several peptide backbone modifications are known, these include, ψ [CH₂S], ψ [CH₂NH], ψ [CSNH₂], ψ [NHCO], ψ [COCH₂] and ψ [(E)]
or (Z) CH=CH]. The nomenclature used above, follows that suggested by Spatola, above. In this context, \( \psi \) indicates the absence of an amide bond. The structure that replaces the amide group is specified within the brackets.

Amino acid mimetics may also be incorporated in the peptides. An "amino acid mimetic" as used here is a moiety other than a naturally occurring amino acid that conformationally and functionally serves as a substitute for an amino acid in a peptide of the present invention. Such a moiety serves as a substitute for an amino acid residue if it does not interfere with the ability of the peptide to elicit an immune response against the appropriate PorA-derived epitope. Amino acid mimetics may include non-protein amino acids, such as \( \beta, \gamma, \delta \)-amino acids, \( \beta, \gamma, \delta \)-imino acids (such as piperidine-4-carboxylic acid) as well as many derivatives of L-\( \alpha \)-amino acids. A number of suitable amino acid mimetics are known to the skilled artisan, they include cyclohexylalanine, 3-cyclohexylpropionic acid, L-adamantyl alanine, adamantylacetic acid and the like. Peptide mimetics suitable for peptides of the present invention are discussed by Morgan and Gainor, (1989) Ann. Repts. Med. Chem. 24:243-252.

As noted above, the peptides employed in the subject invention need not be identical, but may be substantially identical, to the naturally processed PorA-derived antigenic peptides as defined in Table 1. Therefore, the peptides may be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. The peptides of the invention can be modified in a number of ways so long as they comprise a sequence substantially identical (as defined below) to an amino acid sequence of at least one of the naturally processed PorA-derived antigenic peptides selected from Table 1.

TFASTA in the Wisconsin Genetics Software Package Version 10.2, Genetics Computer Group, 575 Science Dr., Madison, Wisconsin 53711, USA), or by inspection, and the best alignment (i.e., resulting in the highest percentage of sequence similarity over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polypeptide sequences are identical (i.e., on an amino acid-by-amino acid basis) over a window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

As applied to the peptides of the invention, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default parameters, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). GAP uses the Needleman and Wunsch global alignment algorithm to align two sequences over their entire length, maximizing the number of matches and minimizes the number of gaps. Generally, the GAP default parameters are used, with a gap creation penalty = 50 (nucleotides) / 8 (proteins) and gap extension penalty = 3 (nucleotides) / 2 (proteins). For nucleotides the default scoring matrix used is nwsgapdna and for proteins the default scoring matrix is Blosum62 (Henikoff & Henikoff, 1992).

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a
group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

5 Pharmaceutical (vaccine) compositions and their administration

Pharmaceutical (vaccine) compositions comprising at least one PorA-derived antigenic peptide of the invention are also an embodiment of the invention.
The peptides of the present invention and pharmaceutical compositions thereof are useful for administration to mammals, particularly humans, to treat and/or prevent Men B infection. Suitable formulations are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985), which is incorporated herein by reference.

The immunogenic peptides of the invention are administered prophylactically or to an individual already suffering from the disease. The compositions are administered to a patient in an amount sufficient to elicit an effective immune response. An amount adequate to accomplish this is defined as "therapeutically effective dose" or "immunogenically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgement of the prescribing physician, but generally range for the initial immunisation (that is for therapeutic or prophylactic administration) from about 0.1 μg to about 50 μg per kilogram (kg) of body weight per patient, more commonly from about 1 μg to about 20 μg per kg of body weight. Boosting dosages are typically from about 1 μg to about 20 μg of peptide using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include 3-4 priming injections at three weeks intervals, eventually followed by booster injections at 26 weeks.

The pharmaceutical compositions are intended for parenteral, oral or transdermal administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the
immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilised by conventional, well known sterilisation techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilised, the lyophilised preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, and triethanolamine oleate.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%. As noted above, the compositions are intended to induce an immune response to the peptides. Thus, compositions and methods of administration suitable for maximising the immune response are preferred. For instance, peptides may be introduced into a host, including humans, linked to a carrier or as a homopolymer or heteropolymer of active peptide units. Alternatively, the a "cocktail" of peptides can be used. A mixture of more than one peptide has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies to a number of epitopes. For instance, peptides comprising sequences from hypervariable regions of α and β chains may be used in combination. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like.
The compositions preferably also include an adjuvant. A number of adjuvants are well known to one skilled in the art. Suitable adjuvants include incomplete Freund's adjuvant, alum, aluminum phosphate, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide. A particularly useful adjuvant and immunisation schedule are described in Kwak et al. New Eng. J. Med. 327-1209-1215 (1992), which is incorporated herein by reference. The immunological adjuvant described there comprises 5% (wt/vol) squalene, 2.5% Pluronic L121 polymer and 0.2% polysorbate in phosphate buffered saline.

The concentration of immunogenic peptides of the invention in the pharmaceutical formulations can vary widely, i.e. from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunisation protocols are described in, e.g., U.S. Pat. No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful for therapeutic administration or immunisation of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.
Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990). Both of these references are incorporated herein by reference in their entirety. E.g. transdermal delivery systems include patches, gels, tapes and creams, and can contain excipients such as solubilisers, permeation enhancers (e.g. fatty acids, fatty acid esters, fatty alcohols and amino acids), hydrophilic polymers (e.g. polycarbophil and polyvinyl pyrrolidone and adhesives and tackifiers (e.g. polyisobutylene, silicone-based adhesives, acrylates and polybutene).

Transmucosal delivery systems include patches, tablets, suppositories, pessaries, gels, and creams, and can contain excipients such as solubilisers and enhancers (e.g. propylene glycol, bile salts and amino acids), and other vehicles (e.g. polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethyl cellulose and hyaluronic acid). Injectable delivery systems include solutions, suspensions, gels, microspheres and polymeric injectables, and can comprise excipients such as solubility-altering agents (e.g. ethanol, propylene glycol and sucrose) and polymers (e.g. polycaprylactones, and PLGA's). Implantable systems include rods and discs, and can contain excipients such as PLGA and polycapryl lactone. Other delivery systems that can be used for administering the pharmaceutical composition of the invention include intranasal delivery systems such as sprays and powders, sublingual delivery systems and systems for delivery by inhalation. For administration by inhalation, the pharmaceutical compositions of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurised packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the peptides of the invention and a suitable powder base such as lactose or starch. The pharmaceutical compositions of the invention may be further formulated for administration by inhalation as e.g. described in U.S. Patent No. 6,358,530.
In another aspect the invention relates to a method for producing a pharmaceutical composition comprising the (poly)peptides of the invention. The method comprises at least the steps of mixing the (poly)peptides of the invention obtained in the methods described above with a pharmaceutically acceptable carrier and further constituents like adjuvant as described above.


Throughout the description and Examples reference to the following sequences is made:

SEQ ID NO 1: amino acid sequence of region 1 PorA peptide P1.5-2,101-18
SEQ ID NO 2: amino acid sequence of region 1 PorA peptide P1.5-2,101-20
SEQ ID NO 3: amino acid sequence of region 2 PorA peptide P1.5-2,1039-54
SEQ ID NO 4: amino acid sequence of region 3 PorA peptide P1.5-2,1055-78
SEQ ID NO 5: amino acid sequence of region 4 PorA peptide P1.5-2,1091-106
SEQ ID NO 6: amino acid sequence of region 4 PorA peptide P1.5-2,1092-106
SEQ ID NO 7: amino acid sequence of region 4 PorA peptide P1.5-2,1093-106
SEQ ID NO 8: amino acid sequence of region 4 PorA peptide P1.5-2,1091-108
SEQ ID NO 9 amino acid sequence of region 5 PorA peptide P1.5-2,1044-163
SEQ ID NO 10: amino acid sequence of region 6 PorA peptide P1.5-2,10318-349
SEQ ID NO 11: amino acid sequence of region 7 PorA peptide P1.5-2,10349-362
SEQ ID NO 12: amino acid sequence of region 7 PorA peptide P1.5-2,10350-362
SEQ ID NO 13 amino acid sequence of region 7 PorA peptide P1.5-2,10349-364
SEQ ID NO 14: amino acid sequence of region 7 PorA peptide P1.5-2,10350-364
SEQ ID NO 15 amino acid sequence of region 7 PorA peptide P1.5-2,10352-367
SEQ ID NO 16: amino acid sequence of region 7 PorA peptide P1.5-2,10353-367
Figure legends

Figures 1a and 1b
Deconvoluted mass spectra of the $^{14}N$-labeled P1.5-2,10 (Fig 1a) and $^{15}N$-labeled P1.5-2,10 (Fig 1b), respectively. Small aliquots of OMV, containing a total protein amount of 40 ng, were subjected to µLC-MS analysis as described (Meiring et al. 2002, J. Sep. Sci. 25, 557-568. 2002). The measured average molecular weight of each protein closely approximates the expected values. A relative increase of 1.17% is observed indicating a uniform $^{15}N$-labeling of the protein.

Figures 2a - 2i
Flow cytometric analysis of a typical large scale culture of immature DC (iDC; Fig 2a and Fig 2c) (dotted lines) and mature DC (mDC; Fig 2b and Fig 2d) (solid lines) dendritic cells (DC) generated from adherent buffy coat cells obtained after leukapheresis.

Figures 2a and 2b: gate-setting in forward-side scatter plots.
Figures 2c and 2d: background FL1/FL2 autofluorescence by iDC and mDC.
Figures 2e - 2i: typical fluorescence patterns on iDC (dotted lines) and mDC (solid lines) of specific markers (dark lines) and isotype-matched controls (grey lines).

Figures 3a - 3c
Mass tag assisted identification of a naturally processed P1.5-2,10 peptide in HLA-DRB1*0101. Peptides were isolated from in vitro cultured human HLA-DRB1*0101 positive DC after pulsing with $^{14}N$ and $^{15}N$ OMV derived from the isogenic Neisserial strain P1.5-2,10 as described in material and methods. Shown are: (Fig3a) the ESI mass spectrum obtained at a retention time of 26.5 minutes containing a triply charged mass spectral doublet at $m/z$ 553.97 and 561.94 amu; (inset) the deconvoluted MH+ spectrum indicating a putative pathogenic peptide containing 24 nitrogen atoms; (Fig 3b) the deconvoluted MS/MS spectrum of this peptide at $m/z$ 553.97 amu revealing a partial sequence (y-ions series, y11-y15) of the P1.5-2,10 originating peptide.
LAGEFGLRALVRANQ; (Fig 3c) the CID mass spectra of its isotopically labeled synthetic analogue, prepared using 2-^{13}C-Glycine-N-Fmoc at the G* position (LAGEFG*TRTAG*RVANQ).

Figure 4

Determination of peptide specificity of a P1.5-2,10 restimulated CD4+ TCL (410/5-2,10) derived from a HLA-DRB1*0101 homozygous donor. IFN\(\gamma\) SFC were measured in Elispot after seeding 2x10^3 410/5-2,10 T cells in the presence of 0.5 \(\mu\)g/ml rP1.5-2,10 (or other PorA serotypes rP1.7,16 and rP1.7-2,4), single 18-mer P1.5-2,10 peptides (12 amino acid-overlapping) at 1 \(\mu\)M, or medium alone.

Figure 5a - 5d

MHC restricted presentation of P1.5-2,10 peptides and protein to CD4+ JS20 T cells. Fig 5a: Recognition by JS20 T cells of 18-mer peptides (10 \(\mu\)M) overlapping the P1.5-2,10_{91-106} region. Fig 5b: Dose dependent recognition by JS20 T cells of the synthetic natural length variants P1.5-2,10_{91-106} (triangles), and P1.5-2,10_{92-106} (squares), and the F95A substitution variant. Fig 5c: Stimulation of JS20 T cell by rP1.5-2,10 after 2 hours uptake and processing by HLA-DR1 positive PBMC. Fig 5d: P1.5-2,10_{92-106} specific proliferation of JS20 T cell is restricted by HLA-DR1 and is blocked in the presence of culture supernatant (1:100) of the HLA-DR specific hybridoma B8.11.2, but not of the HLA-DQ specific hybridoma SPV-L3 (data not shown).

Figure 6

Hypothetical fit of the P1.5-2,10_{91-106} epitope in the HLA-DR1 binding groove. Shown are proposed major (\(\downarrow,\uparrow\)) and minor (\(\downarrow,\uparrow\)) MHC- or TCR contact residues, based on the crystal structure of HLA-DR1 (Stern et al. 1994, Nature 368, 215-221) and the effect on JS20 T cell recognition of length and substitution variants (summarised in Table 2).
1. Material and methods

1.1. Growth of neisserial P1.5-2,10 strain in minimal medium and OMV preparation

The construction of a class 3-, class 4- isogenic H44/76 strain expressing the serosubtype P1.5-2,10 was described previously (Peeters et al. 1996, *Vaccine* 14, 1009-1015). For stable isotope protein labeling the P1.5-2,10 strain was grown until stationary phase, either in natural $^{14}$NH$_4$Cl containing minimal meningococcal medium, or in >98% enriched $^{15}$NH$_4$Cl stable isotope (Spectra Stable Isotopes, Columbia, USA) containing minimal meningococcal medium, respectively (Jyssum K. Assimilation of nitrogen in meningococci grown with the ammonium ion as sole nitrogen source. Acta Path.Microbiol.Scand. 46, 320-332. 1959). From these cultures batches of $^{14}$N- and $^{15}$N-OMV were prepared and characterized according to Claassen et al. (Claassen et al. 1996, *Vaccine* 14, 1001-1008), and subjected to mass spectrometric analysis (Meiring et al. 2002, A. P. Nanoscale LC-MS$^{(0)}$: technical design and applications to peptide and protein analysis. J.Sep.Sci. 25, 557-568.

1.2. Isolation of PBMC

Peripheral blood mononuclear cells (PBMC) from HLA-oligotyped donors after leukapheresis were isolated by centrifugation of buffy coat cells on ficoll-hypaque (Pharmacia Biotech, Uppsala Sweden), and were freshly used or cryopreserved until usage in experiments.

1.3. Dendritic cell culture, antigen pulse and characterization

Immature CD83$^+$, HLA-DR$^+$ human DC were cultured according to a procedure by Sallusto et al. (Sallusto & Lanzavecchia 1994, *J. Exp. Med.* 179, 1109-1118). Briefly, 1x10$^9$ PBMC were freshly isolated from a HLA-DRB1*0101 homozygous donor, and seeded at ~5x10$^6$ PBMC/ml in 150 mm tissue culture dishes (Costar) in Iscove’s Modified Dulbecco’s Medium (Gibco) supplemented with 1% FCS at 37 °C, 5% CO$_2$, in a humidified incubator, for 2 hr. After removal of the non-adherent fraction, adherent cells were further cultured for 6 days in medium containing 500 U/ml recombinant human Granulocyte-Macrophage Colony Stimulating Factor (rhGM-CSF, PeproTech) and 250 U/ml recombinant human Interleukin-4 (rhIL-4, Strathman Biotech). Culture
medium and growth factors were refreshed on day 3. On day 6 immature DC were pulsed with a mixture of \(^{14}\text{N}\)-P1.5-2,10 OMV and \(^{15}\text{N}\)-P1.5-2,10 OMV (at a protein ratio of 1:1) at a total protein concentration of \(-0.02 \text{ mg/ml}\), and incubated for 6 hr. Hereafter cells were further cultured and matured in the continuous presence of OMV (~0.01 mg protein/ml), 250 U/ml rhGM-CSF, 125 U/ml rhIL-4, and 20 ng/ml LPS (\(S. abortis equi\), Sigma). On day 8 mature OMV pulsed DC were harvested, washed in PBS containing 2.0 mM EDTA, counted, pelleted, snap frozen on dry ice and held in \(-135^\circ\text{C}\). Typically the protocol yields 50-90x10\(^6\) mature DC from 1x10\(^9\) PBMC. Small aliquots of immature and mature OMV pulsed DC were characterised by flow cytometry, using FITC-conjugated or PE-conjugated anti-human mAbs specific for HLA-DR (Sigma clone HK14), CD80 (SeroTec), CD86 (PharMingen), CD83 (PharMingen), CD40 (PharMingen), and appropriate isotype matched controls. After staining samples were washed with cold FACS buffer and approximately 10,000 events were acquired in the FSC-SSC gate for large cells on a FACSCalibur\textsuperscript{TM} flow cytometer (Becton Dickinson, USA) and analysed using CELLQuest\textsuperscript{TM} software (Becton Dickinson, USA).

1.4 Peptide synthesis

Synthetic peptides for T cell studies were prepared by standard solid phase Fmoc chemistry using an ABIMED AMS 422 simultaneous multiple peptide synthesiser (ABIMED Analysen-Technik GmbH, Langenfeld, Germany). Heavy isotope labeled variants of peptides for MS purposes were synthesised using (99 atom\%) \(^{13}\text{C}\)-Glycine-N-Fmoc at the G\(^*\) position (ARC laboratories, Apeldoorn, The Netherlands).

1.5 HLA-DR associated peptide isolation, and \(\mu\text{LC-ESI-MS}\) mass spectrometry

HLA-DRB1*0101 molecules were immunoprecipitated from OMV pulsed DC, essentially as described previously (van Els et al. 2000 \textit{Eur. J. Immunol.} 30, 1172-1181), using the HLA-DR specific monoclonal antibody B8.11.2 bound to Protein A beads. Peptides were eluted using 10% acetic acid, and spun through a 10 kD cutoff spinfilter (Millipore). Peptide analysis of 1-10x10\(^6\) cell equivalents (ce) of sample was performed using microcapillary liquid chromatography electrospray ionisation mass spectrometry (\(\mu\text{LC-ESI-MS}\)) (Meiring et al. 2002, J. Sep. Sci. 25, 557-568) on an ion trap
instrument LCQ Classic (Finnigan, MAT, San Jose, CA, USA), and on a QTOF-Ultima
instrument (Micromass, Almere, the Netherlands, resolution 11,000 amu). Mass spectra
were searched for characteristic $^{14}$N/$^{15}$N ion doublet formation of OMV derived peptides.
Candidate OMV derived $^{14}$N ions or their $^{15}$N counterparts were selected for MS
sequencing at optimized Collision Activated Dissociation (CAD) energy. The sequence of
the allocated peptides was confirmed using synthetic peptides. For quantitation of the
amount of a peptide in a sample, peptide mixtures were spiked with known amounts of
two synthetic peptide standards (Angiotensin III and Oxytocin, Sigma-Aldrich, St
Louis, MO, USA) directly after peptide isolation to correct for sample loss during the
subsequent processing of the samples, assuming equal specific responses (counts per
pmol) and equal losses for peptides and internal synthetic standards.

1.6 Generation of PorA specific CD4+T cell lines (TCL)
PorA$_{91-106}$ and PorA$_{349-367}$ specific TCL were generated from HLA-DRB1*0101 positive
donors after culturing of PBMC ($2 \times 10^5$ cells/well) in 96-well round bottom plates in
AIM-V medium in the presence of 2% human serum and 2-20 µM of synthetic peptide
or 0.5 µg/ml rPorA. Ten days after the initiation of the cultures 10 U/ml of recombinant
Interleukin 2 (rIL-2, Cetus Corp., Emerville, CA, USA) was added. TCL were
repetitively restimulated at 14-21 days intervals, using $2 \times 10^5$ peptide- or rPorA pulsed,
irradiated autologous or HLA-DR matched PBMC and rIL-2.

1.7 T cell proliferation assay
Proliferation assays were performed as described by Van Bleek et al. (Van Bleek et al.
2003, *J. Virol.* 77, 980-988). Briefly, either PBMC ($10^5$/well), TCL ($2 \times 10^4$/well,
supplemented with $10^5$ irradiated autologous PBMC/well) from HLA-DRB1*0101
positive individuals were cultured in 96-well roundbottom microculture plates in AIM-
V medium (Gibco) containing 2% human serum in the presence of either no antigen,
synthetic peptide, or recombinant PI.5-2,10 (rPI.5-2,10) (or other rPorA serosubtypes,
generously provided by S. Pillai, Wyeth Lederle, West Henrietta, USA), at indicated
doses. In blocking experiments irradiated PBMC were preincubated in the presence or
absence of culture supernatant (1:100) of the HLA-DR specific hybrididoma B8.11.2 or
the HLA-DQ specific hybridoma SPV-L3 before addition of antigen and coculture with TCL. After 5 days (for PBMC) or 2 days (for TCL) the cultures were pulsed with 0.5 μCi of [3H]thymidine (Amersham, UK). Sixteen hours later cultures were harvested on filters using a 96-well sample harvester (LKB, Wallac, Turku, Finland) and cpm were determined using a liquid scintillation β-counter (LKB, Wallac, Turku, Finland). Results obtained from triplicate or six replicate cultures were represented as stimulation index (S.I.) as follows: S.I. = (mean cpm obtained in the presence of antigen presenting cells and antigen)/(mean cpm obtained in the presence of antigen presenting cells and medium only).

1.8 Elispot assay

IFNγ Elispot assay were performed according to Van Bleek et al. (supra). Briefly, 96 well filtration plates (MAIPS4510, Millipore) were coated overnight with anti-IFN-γ coating antibody (clone 1-D1K, Mabtech) in carbonate buffer at 4°C. After washing and blocking of the plates 2x10^5 PBMC or 2x10^3 TCL or TCC per well were seeded in culture medium in the presence or absence of antigen, as indicated, and for TCL supplemented with 2x10^5 irradiated autologous or HLA-DR matched PBMC, for a 24 hour incubation at 37°C, 5% CO2, in a humidified incubator. Then, plates were washed, developed with chromogenic alkaline phosphatase substrate after a two-step incubation with biotinylated anti-IFNγ detecting antibody (clone 7-B6-1, Mabtech) and streptavidin-alkaline phosphatase, and the number of spotforming cells (SFC) per well was counted using AELVIS hardware and software (AELVIS GmbH, Hannover, FRG). Responses were considered positive if the number of SFC/well was five or more and at least twice that in wells containing medium or control antigen.

2. Results

2.1 Preparation of 14N- and 15N-labeled outer membrane vesicles from a neisserial isogenic strain expressing P1.5-2.10

PorA munts to approximately 90% of the protein content of experimental OMV based Men B vaccines isolated after deoxycholate extraction and purification of heat-
inactivated meningococci (Claassen et al. 1996, Vaccine 14, 1001-1008). To allow synthesis of stable heavy and light forms of meningococcal outer membrane proteins, 1 L log-phase cultures of the class 3', class 4' isogenic H44/76 strain expressing the PorA serosubtype P1.5-2,10 were prepared either in 14N-containing medium, or in 98% 15N-atom enriched medium. OMV prepared from these cultures contained 2 and 1.7 mg protein/ml, respectively, and had identical PorA migration patterns in SDS-PAGE (data not shown). μLC-MS analysis of the contents of 15N-heavy and 14N-light OMV identified a mass increment (1.18%) for the heavy form of P1.5-2,10 relative to the light form of the P1.5-2,10 protein (Figure 1). In addition, the MS/MS spectra obtained from trypsin treated heavy and light OMV revealed typical fragmentation of P1.5-2,10 digest products into 15N-heavy and 14N-light forms of amino acids, respectively (data not shown), confirming the successful stable isotope labeling throughout the full sequence of the P1.5-2,10 protein.

2.2 Loading of cultured human HLA-DR1 homozygous dendritic cells with stable isotope labeled P1.5-2,10 OMV

To study antigen presentation relevant for T cell priming we applied an in vitro culture system for the outgrowth of human monocyte-derived immature DC for antigen uptake and processing (Sallusto & Lanzavecchia 1994, J. Exp. Med. 179, 1109-1118). Adherent buffy coat cells obtained from a homozygous HLA-DRB1*0101 typed donor were cultured in medium supplemented with rhGM-CSF and rhIL-4. After 6 days DC are still immature and appear as loosely adherent clumps or as isolated floating cells with typical dendritic morphology (data not shown). At this stage a 1:1 (protein ratio) mixture of (15N) heavy and (14N) light P1.5-2,10 OMV (containing traces of N. meningitidis LPS) was fed to the cultures and antigen processing and DC maturation was allowed for 48 hr in the presence of rhGMC-SF, rhIL-4 and LPS (S. abortis equi). At day 8 adherent and floating DC were collected and washed extensively before MHC peptide isolation. Exposure of immature DC to OMV and LPS induced DC maturation as was illustrated by the upregulation of DC marker CD83 and the co-stimulatory molecules CD80 and CD40, while expression of HLA-DR and CD86 remains relatively unchanged (Figure 2). These phenotype changes are similar to those described for clinical grade DC (de Vries 2002, J. Immunother. 25, 429-438).
2.3 Mass tag assisted identification of naturally processed and HLA-DR presented P1.5-2,10 derived epitopes

As an example, HLA-DR1 (B1*0101) molecules were purified from \(^{14}\text{N}\) and \(^{15}\text{N}\) OMV pulsed DC, bound peptides were eluted and separated by size exclusion, and aliquots were analysed by \(\mu\)LC-ESI-MS\(^{60}\). The total ion chromatogram recorded in data dependent mode indicates the enormous complexity of the sample (data not shown). Extracted and deconvoluted MH\(^+\) peptide spectra were searched for the appearance of \(^{14}\text{N}\)- and \(^{15}\text{N}\)-ion doublets, recognised as paired ion signals with similar intensity and retention time, and an average mass difference of 1.17\%. Of eight ion doublets detected, six potentially represented P1.5-2,10 derived \(^{14}\text{N}\)- and \(^{15}\text{N}\) peptide pairs, based on Mw and the number of N-atoms. An example is shown in Figure 3 (top). The MS/MS spectrum recorded for the \(^{14}\text{N}\) [M+H]\(^+\) ion of the 1659.89/1683.82 doublet revealed homology with amino acid residues 91-106 of P1.5-2,10 (LAGEFGLRAGRVANQ) (Figure 3, middle section). This match was confirmed by comparison with the fragmentation pattern of a synthetic standard which was \(^{13}\text{C}\) isotopically labelled to avoid contamination of the elution sample (Figure 3, bottom).

In total, the mass tag assisted approach revealed 10 naturally processed and HLA-DRB1*0101 presented epitopes derived from 4 regions of the P1.5-2,10 protein, expressed at diverging densities per cell (Table 1). Likewise, 8 naturally processed and HLA-DRB1*1501 presented epitopes derived from 4 regions of the P1.5-2,10 protein were identified, which partially overlapped the HLA-DRB1*0101 presented epitopes (Table 1). At the applied detection limit of the MS system (100 c/c), no other epitopes or length variants were detected. Table 1 also includes a peptide comprising a naturally processed PorA derived epitope presented by HLA-DRB1*07 molecules (Seq ID NO: 4).

2.4 CD4\(^+\) T cell reactivity to PorA\(_{91-108}\) in HLA-DR1 positive individuals

To assess whether the four naturally processed HLA-DRB1*0101 presented P1.5-2,10 regions represent human meningococcal CD4\(^+\) T cell epitopes, PBMC from three HLA-DR1 positive healthy blood donors were tested \textit{ex vivo} in a \(^{3}\text{H}\) thymidine proliferation assay using overlapping synthetic peptides (18 aa) encompassing these
sequences. A weak direct specific proliferative response was measured in at least two out of three donors against all four regions. Repetitive in vitro stimulation of HLA-DR1 positive PBMC with peptides representing P1.5-2,1091-106 and P1.5-2,10349-367, but not P1.5-2,101-18, led to the outgrowth of short term peptide specific CD4+ T cell lines, usually also displaying weak reactivity against autologous rP1.5-2,10 pulsed APC (data not shown). Further support for the immunogenicity of the P1.5-2,1091-106 and P1.5-2,10349-367 regions came from studies using a CD4+ bulk culture (410/5-2,10) established from PBMC from an HLA-DR1 homozygous donor after 3-5 repetitive in vitro restimulations with autologous rP1.5-2,10 pulsed APC, allowing any rP1.5-2,10 derived HLA-DR1 epitopes to be presented at naturally processed levels. 410/5-2,10 CD4+ T cells proliferated specifically against rP1.5-2,10, and showed fine specificity uniquely against P1.5-2,1091-106 and P1.5-2,10349-367 when tested against a full protein pepscan in IFNγ ELISPOT (Figure 4).

To elaborate on the molecular requirements of P1.5-2,10 recognition, a long term monoclonal CD4+ T cell line (JS20), specific for the P1.5-2,1091-106 region (Figure 5a), was functionally tested in more detail. JS20 T cells responded to the two naturally occurring variants, P1.5-2,1091-106 and P1.5-2,1092-106, with comparable dose-response rates (Figure 5b). To confirm that recognition of P1.5-2,10 epitopes by JS20 T cells occurred after natural antigen processing in the context of HLA-DR1, autologous, HLA-DR1 matched or -mismatched PBMC were allowed to process rP1.5-2,10 for a minimum of two hours before irradiation and coculturing for 48 hr with JS20 T cells. Proliferation of JS20 T cells was induced by processed rPorA in a dose dependent manner (Figure 5c), required the presence of the HLA-DR1 allele, and was inhibitable by the addition of HLA-DR blocking mAbs (Figures 5d). Finally, the effects of N-and C-terminal truncations and single amino acid (aa) substitutions within the P1.5-2,1091-106 sequence on T cell recognition were investigated (data summarized in Table 2). A minimal core length of of 11 aa (P1.5-2,1094-104) was required for preservation of JS20 T cell proliferation or IFNγ production. In this sequence alanine or charge substitutions at positions E94, F95, T97, L98, R99, G101, R102, and V103 clearly diminished or abolished the ability to stimulate JS20 activity. From these analyses hypothetical contact residues
important for the fit of the P1.5-2,1091-106 epitope into the HLA-DR1 binding groove and for interaction with the JS20 TCR can be proposed (Figure 6).

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*PorA residue numbering according to the appearance in the P1.5-2,10 protein chain after cleavage of the N-terminal signal peptide
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*Shown is the relative responsiveness of JS20 T cells in a proliferative assay in the presence of synthetic length- and amino acid substitution variants of P1.5-2,1091-106 at 1 μM. Cpm in the presence of medium, P1.5-2,1091-106 and P1.5-2,1092-106 were 137, 39498 and 34767, respectively. Identical trends were observed in IFNγ ELISPOT (data not shown).
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Glu Glu Gly Gly Leu Asn Leu Al a Leu Ala Ala Gln Leu Asp Leu Ser
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265  
270  

Glu Asn Ala Asp Lys Thr Lys Asn Ser Thr Thr Glu Ile Ala Ala Thr
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280  
285  

Ala Ser Tyr Arg Phe Gly Asn Ala Val Pro Arg Ile Ser Tyr Ala His
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295  
300  

Gly Phe Asp Phe Ile Glu Arg Gly Lys Lys' Gly Glu Asn Thr Ser Tyr
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310  
315  
320  

Asp Gln Ile Ile Ala Gly Val Asp Tyr Asp Phe Ser Lys Arg Thr Ser
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Ala Ile Val Ser Gly Ala Trp Leu Lys Arg Asn Thr Gly Ile Gly Asn
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345  
350  

Tyr Thr Gln Ile Asn Ala Ala Ser Val Gly Leu Arg His Lys Phe
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360  
365  

35
Claims

1. A method for ex vivo diagnosis of human MHC class II restricted immune responses to Neisseria meningitidis serogroup B antigens in a subject, wherein the method comprises the steps of:
   (a) determining the MHC class II haplotype of the subject;
   (b) providing a composition comprising peripheral blood mononuclear cells (PBMC's) from the subject;
   (c) mixing the composition comprising PBMC's with a soluble peptide comprising at least a naturally processed PorA-derived antigenic peptide sequence selected from Table 1 or a fragment, variant, or HLA-peptide multimer derived from the antigenic peptide sequence, whereby the antigenic peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1, or a cross-presenting variant thereof, and,
   (d) determining the presence of PorA specific MHC class II restricted immune cells in the subject's PBMC's.

2. A method according to claim 1, wherein in step (d) the proliferation of PBMC's is determined.

3. A method according to claim 2, wherein the proliferation of T cells is determined.

4. A method according to claim 2, wherein the proliferation of T cells is determined without pre-expansion of the T cells.

5. A method according to claim 3 or 4, wherein the proliferation of CD4+ T cells is determined.

6. A method according to claim 1, wherein in step (d) the effector cell function of CD4+ T cells is determined by measuring cytokine secretion or production.
7. A method according to claim 6, wherein IFN-γ production is measured in a (direct) ELISPOT assay.

8. A method according to any one of claims 1 - 7, wherein in step (c) the soluble peptide is mixed with the preparation of PBMC's at a concentration of a least 5 nM.

9. A method according to claim 1, wherein in step (d) PorA specific MHC class II restricted immune cells are quantitated by staining with fluorescent HLA-peptide multimers.

10. A method according to claim 1, wherein in step (d) PorA specific MHC class II restricted CD4 T cells are quantitated by staining with fluorescent HLA-peptide multimers.

11. A method according to any one of claims 1 - 10, wherein the subject has undergone or is undergoing an immune responses to *N. meningitidis* serotype B.

12. A method according to any one of claims 1 - 10, wherein the subject has been vaccinated against *N. meningitidis*.

13. Use of a method according to claim 1 - 10, to evaluate correlates of protection in vaccinated individuals.

14. A method for haplotype specific vaccination of a subject against *N. meningitidis* serotype B, the method comprising the steps of:
(a) determining the MHC class II haplotype of the subject, and
(b) administering to the subject a pharmaceutical composition comprising a peptide or protein comprising at least one naturally processed PorA-derived antigenic peptide sequence selected from Table 1, or a variant of the peptide sequence, whereby the peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1 or a cross-presenting variant thereof.
15. A method according to claim 14, wherein the pharmaceutical composition is suitable for parenteral administration and is administered parenterally, or wherein the pharmaceutical composition is suitable for transdermal administration and is administered transdermally.

16. Use of a peptide or protein comprising a naturally processed PorA-derived antigenic peptide sequence selected from Table 1, or a variant of the peptide sequence, for the manufacture of a vaccine for prophylaxis or therapy of *N. meningitidis* serotype B infection in a subject.

17. A use according to claim 16, wherein the antigenic peptide sequence matches the MHC class II haplotype of the subject in accordance with Table 1.

18. A use according to any one of claims 16-17, wherein the vaccine is a pharmaceutical composition suitable for parenteral or transdermal administration.

19. A pharmaceutical composition comprising peptide or protein comprising a naturally processed PorA-derived antigenic peptide sequence selected from Table 1, or a variant of the peptide sequence.
Fig 1a

14-N-P1,5,2,10 PorA

Measured: Mw<sub>av</sub> = 39,769 amu
Theoretical: Mw<sub>av</sub> = 39,786 amu

Relative Abundance

Fig 1b

15-N-P1,5,2,10 PorA

Measured: Mw<sub>av</sub> = 40,273 amu
Theoretical: Mw<sub>av</sub> = 40,278 amu

Relative Abundance
Fig 5b

Fig 5c

Fig 5d
Fig 6

\[
\uparrow \uparrow \uparrow \uparrow \uparrow \quad \text{TCR}
\]

\[
\text{(L}^{91}) \text{AGEFGTLRAGRVANQ}^{106}
\]

\[
\downarrow \downarrow \downarrow \downarrow \quad \text{MHC}
\]

p 1 4 7 9