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(AT). **LINGNAU, Karen** [DE/AT]; Gallgasse 8/10,
A-1130 Wien (AT).

(74) **Agent: BÖSL, Raphael;** Isenbruck Bosl Horschler Wich-
mann Huhn, Prinzregentenstrasse 68, 81675 Munich (DE).

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(71) **Applicant** (for all designated States except US): **INTER-
CELL AG** [AT/AT]; Campus Vienna Biocenter 6, A-1030
Vienna (AT).

(72) Inventors; and

(75) **Inventors/Applicants** (for US only): **NAGY, Eszter**
[HU/AT]; Klimschgasse 30/11/21, A-1030 Vienna (AT).
MEINKE, Andreas [DE/AT]; Piettegasse 26/1, A-3013
Pressbaum (AT). **VON GABAIN, Alexander** [AT/AT];
Hockegasse 77, A-1 180 Vienna (AT). **HANNER, Markus**
[AT/AT]; Terrassengasse 12, A-3021 Pressbaum (AT).
NOIGES, Birgit [AT/AT]; Schönbrunnerstrasse 100/20,
A-1050 Vienna (AT). **GELBMANN, Dieter** [AT/AT];
Ungergasse 5, A-7163 Andau (AT). **SENN, Beatrice**
[CH/AT]; Gumpendorferstrasse 135/41, A-1060 Vienna

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(54) **Title:** *NEISSERIA MENINGITIDIS* ANTIGENS

(57) **Abstract:** The present invention relates to a peptide consisting of antigen of *N. meningitidis* of any of the SEQ ID NOS: 1 to 184 or a functional active fragment or derivative thereof, optionally further consisting of additional amino acid residue(s); a nucleic acid coding for the same; a pharmaceutical composition, especially a vaccine, containing a polypeptide comprising or consisting of said peptide or said nucleic acid; a nucleic acid coding for said polypeptide or a nucleic acid complementary to the same; an antibody or functional active fragment thereof; a hybridoma cell line which produces said antibody; a method for producing said antibody; a pharmaceutical composition comprising said antibody; the use of said peptide or said nucleic acid for the manufacture of a medicament for the immunization of a subject; the use of said antibody or functional fragment thereof for the manufacture of a medicament for the treatment of an infection; a method of diagnosing a *N. meningitidis* infection; a method for identifying a ligand capable of binding to said peptide; and the use said peptide for the isolation and/or purification and/or identification of an interaction partner of the peptide.

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Neisseria meningitidis Antigens

- 5 The present invention relates to a peptide consisting of one antigen of *N. meningitidis* of any of the SEQ ID NOS: 1 to 184 or a functional active fragment or derivative thereof, optionally further consisting of additional amino acid residue(s); a nucleic acid coding for the same; a pharmaceutical composition, especially a vaccine, containing a polypeptide comprising or consisting of said peptide or said nucleic acid; a nucleic acid coding for said
- 10 polypeptide or a nucleic acid complementary to the same; an antibody or functional active fragment thereof; a hybridoma cell line which produces said antibody; a method for producing said antibody; a pharmaceutical composition comprising said antibody; the use of said peptide or said nucleic acid for the manufacture of a medicament for the immunization of a subject; the use of said antibody or functional fragment thereof for the
- 15 manufacture of a medicament for the treatment of an infection; a method of diagnosing a *N. meningitidis* infection; a method for identifying a ligand capable of binding to said peptide; and the use of said peptide for the isolation and/or purification and/or identification of an interaction partner of the peptide.
- 20 *Neisseria meningitidis* is a leading cause of purulent meningitis and septicemia world-wide and affects predominantly infants between 6 months and 2 years of age as well as teenagers. Meningococcal meningitis and sepsis are devastating diseases that can kill children and young adults within hours despite the availability of effective antibiotics.
- 25 The disease is caused by *Neisseria meningitidis*, or meningococcus, an aerobic gram-negative diplococcus, that is closely related to *Neisseria gonorrhea*, and to several nonpathogenic *Neisseria* species, such as *N. lactamica*. The organism has both an inner (cytoplasmic) and outer membrane, separated by a cell wall. The outer membrane contains several protein structures, which enable the bacteria to interact with the host cells, as well
- 30 as other functions. The outer membrane is surrounded by a polysaccharide capsule that is necessary for pathogenicity because it helps the bacteria to resist phagocytosis and

complement-mediated lysis. The outer membrane proteins and the capsular polysaccharide comprise the main surface antigens of the organism. Meningococci are classified using serological methods based on the structure of the polysaccharide capsule. Thirteen antigenically and chemically distinct polysaccharide capsules have been described. Some strains, often those found to cause asymptomatic nasopharyngeal carriage, are not groupable, and do not have a capsule. Almost all invasive disease is caused by one of five serogroups: A, B, C, Y, and W-135. The relative importance of each serogroup depends on geographic location, as well as other factors, such as age. The serogroup B meningococcus is responsible for the majority of cases of meningococcal disease in temperate countries.

Meningococcal disease may manifest itself as either a systemic infection of the bloodstream (meningococcaemia) or an inflammation of the meninges of the brain and spinal cord (meningitis). Meningitis is the most common presentation of invasive meningococcal disease and results from hematogenous dissemination of the organism. Meningeal infection is similar to other forms of acute purulent meningitis, with sudden onset of fever, headache, and stiff neck, often accompanied by other symptoms, such as nausea, vomiting, photophobia (eye sensitivity to light), and altered mental status. Meningococci can be isolated from the blood in up to 75 % of persons with meningitis. Meningococcal sepsis (bloodstream infection or meningococcemia) occurs without meningitis in 5 % - 20 % of invasive meningococcal infections. Less common presentations of meningococcal disease include pneumonia (5 % - 15 % of cases), arthritis (2 %), otitis media (1 %), and epiglottitis (< 1 %). The case fatality rate of invasive meningococcal disease is 9 % - 12 %, even with appropriate antibiotic therapy. The fatality rate of meningococcemia is up to 40 %. Up to 20 % of survivors have permanent sequelae, such as hearing loss, neurologic damage, or loss of a limb.

Meningococcal disease occurs worldwide in both endemic and epidemic form. The reported annual incidence of meningococcal disease varies from 0.5 to 10 per 100,000 persons. However, during epidemics the incidence can rise above 400 per 100,000. The case fatality rate ranges from 5 % to 15 %, and up to 25 % of survivors are left with neurological sequelae. Recently, 2,600 and 5,600 cases were reported annually in the United States and Europe, respectively.

Humans are the only natural reservoir of meningococcus. Up to 10 % of adolescents and adults are asymptomatic transient carriers of *N. meningitidis*, most of which are not pathogenic (i.e., strains that are not groupable). Primary transmission mode is by respiratory droplet spread or by direct contact. The communicability of *N. meningitidis* is generally limited. In studies of households in which a case of meningococcal disease has occurred, only 3 % - 4 % of households had secondary cases. Most households had only one secondary case. Estimates of the risk of secondary transmission are generally 2-4 per 1,000 household members at risk. However, this risk is 500-800 times that of the general population.

Serum bactericidal activity is considered to be the major protective immune mechanism and the most important parameter to be analysed when testing antigens to be used in vaccines.

Meningococcal infection kills 50 % of untreated patients and 5 % - 15 % of patients who receive currently available treatments, according to the National Center for Genome Resources in the US. Since the vast majority of strains are susceptible to Penicillin, it is the primary choice of treatment.

A vaccine can contain a whole variety of different antigens. Examples of antigens are whole-killed or attenuated organisms, subfractions of these organisms/tissues, proteins, or, in their most simple form, peptides. Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T-cells (CTL) recognize antigens in form of short usually 8 - 11 amino acids long peptides in conjunction with major histocompatibility complex (MHC). B-cells can recognize linear epitopes as short as 4 - 5 amino acids, as well as three-dimensional structures (conformational epitopes). In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system. Primarily, adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APCs). These cells usually first encounter the antigen(s) followed

by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antigens and co-injected other factors. In addition, the adjuvant may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

In the 1960s, vaccines consisting of purified polysaccharide antigens were developed against four (A, C, Y, and W135) of the five pathogenic serogroups. These vaccines are highly effective in adults but are not efficacious in infants and young children, the age groups mostly exposed to disease. Second-generation glycoconjugate vaccines that are also effective in infants and children are now in the later phases of development. Currently, there are no vaccines available for prevention of serogroup B *N. meningitidis* (MenB) disease, which is responsible for 32 % of all meningococcal disease in the United States and for 45 % to 80 % of the cases in Europe. The use of capsular polysaccharide as the basis of a vaccine for prevention of MenB diseases has been problematic. The MenB capsular polysaccharide is identical to a widely distributed human carbohydrate [a(238)7V-acetyl neuraminic acid or polysialic acid], which, being a self-antigen, is a poor immunogen in humans. Furthermore, use of this polysaccharide in a vaccine may elicit autoantibodies. An alternative approach to vaccine development is based on surface-exposed proteins contained in outer membrane vesicles (OMVs). These vaccines have been shown both to elicit serum bactericidal antibody responses and to protect against developing meningococcal disease. However, sequence variation of most of the surface-exposed proteins (such as PorA, PorB), current targeted, hampered efforts to develop a successful vaccine. There remains a need in the art for the development of antigens from *N. meningitidis* serogroup B and peptides comprising the same which are useful in research, diagnosis and treatment of infections.

Accordingly, it was one object of the present invention to provide such antigens and peptides.

Surprisingly, the object has been solved by a peptide consisting of one antigen of *N. meningitidis* of any of the SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183 and/or 184 or a functional active fragment thereof. These peptides are referred to as antigenic peptides.

The fragment is characterized by being derived from the antigen as defined above by one or more deletions. The deletion(s) may be C-terminally, N-terminally and/or internally. Preferably the fragment is obtained by 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably 1, 2, 3, 4 or 5, even more preferably 1, 2 or 3, still more preferably 1 or 2, most preferably 1 deletion(s). The functional active fragment of the invention is characterized by having a biological activity similar to that displayed by the complete protein, including the ability to exhibit reactivity with Men B specific antibodies, to mediate seroconversion and/or to show bactericidal activity. The fragment of an antigen is functional active in the context of the present invention, if the activity of the fragment amounts to at least 10 %, preferably at least 25 %, more preferably at least 50 %, even more preferably at least 70 %, still more preferably at least 80 %, especially at least 90 %, particularly at least 95 %, most preferably at least 99 % of the activity of the antigen without sequence alteration. These fragments may be designed or obtained in any desired length, including as small as about 5 to 8 amino acids in length.

The sequences of SEQ ID NOS: 1 to 184 are characterized in tables 3 to 5 of the present specification disclosing the underlying amino acid sequences and their immunologic properties. The peptides of SEQ ID NOS: 1 to 184 have been shown to exhibit reactivity

with Men B specific antibodies, such as with human sera of convalescing Men B and optionally Men C patients or their family members (see Example 1 and 3).

Preferably, the antigenic peptide is also able to mediate seroconversion. Seroconversion in the context of the present invention refers to the development of detectable specific antibodies to the antigenic peptide in the serum as a result of infection or immunization. Examples of such antigenic peptides exhibiting strong seroconversion are those consisting of the SEQ ID NO: 1 to 55. Examples of antigenic peptides exhibiting weak seroconversion are those consisting of the SEQ ID NO: 56 to 94. Seroconversion may be assessed by calculating the fold increase of antibody levels in convalescent samples relative to that in acute sera (e.g. > 2 fold). Methods for determining antibody levels and seroconversion are known to the person skilled in the art and described below (see e.g. Example 3).

Even more preferably, the antigenic peptides show bactericidal activity. Bactericidal activity in the context with the present application refers to the ability of a peptide of inducing immunofunctional antibodies against *N. meningitidis* serogroup B. Bactericidal activity can be tested with epitope-induced hyperimmune mouse sera. Such a test for determining bactericidal activity is e.g. described in Example 4.

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In another preferred embodiment of the invention the peptide of the invention can further consist of

- (i) 1 to 50 additional amino acid residue(s), preferably 1 to 40, more preferably 1 to 30, even more preferably at most 1 to 25, still more preferably at most 1 to 10, most preferably 1, 2, 3, 4 or 5 additional amino acids residue(s); and/or
- (ii) at least one amino acid residue heterologous to the antigen.

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The antigen of *N. meningitidis* can be any of the antigens as defined above, particularly as defined in any of the SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89,

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90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 5 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183 and/or 184 or a functional active fragment thereof. Preferred are those as defined in any of the SEQ ID NOS: 1 to 94 or 110 to 184, more preferred those as defined in any of the SEQ ID NOS: 1 to 94 and most preferred those as defined in any of the SEQ ID NOS: 1 to 55 or a functional active fragment thereof. The functional active fragment is as defined 10 above.

The additional amino acid residue(s) of (i) and/or (ii) may be any amino acid, which may be either an L-and/or a D-amino acid, naturally occurring and otherwise. Preferably the amino acid is any naturally occurring amino acid such as alanine, cysteine, aspartic acid, 15 glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan or tyrosine.

However, the amino acid may also be a modified or unusual amino acid. Examples of those are 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, 2-aminobutyric acid, 4- 20 aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-diaminobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycinem N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproloine, 4-hydroxyproloine, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, 6-N-Methyllysine, N-methylvaline, 25 norvaline, norleucine or ornithine. Additionally, the amino acid may be subject to modifications such as posttranslational modifications. Examples of modifications include acetylation, amidation, blocking, formylation, γ -carboxy glutamic acid hydroxylation, glycosilation, methylation, phosphorylation and sulfatation. If more than one additional or heterologous amino acid residue is present in the peptide, the amino acid residues may be 30 the same or different from one another.

In one embodiment the peptide of the invention further encompasses at least one amino acid residue heterologous to the antigen. The feature "heterologous amino acid" or "amino acid heterologous to the antigen" refers to any amino acid which is different from that amino acid located adjacent to the antigen in any naturally occurring protein of *N. meningitidis*, particularly from that of *N. meningitidis* B, especially that of *N. meningitidis* B strain MC58. Therefore, the protein of the invention encompassing at least one heterologous amino acid refers to a protein which is different from any naturally occurring protein of *N. meningitidis* or fragment thereof, particularly which is different from that of *N. meningitidis* B, especially strain MC58. The full length genome of *N. meningitidis* B strain MC58 and the encoded proteins are disclosed e.g. in WO 00/022430.

In one preferred embodiment of the invention the peptide of the invention comprising additional amino acid residue(s) as defined above is characterized in that it comprises at least 2, preferably at least 3 antigens as defined above.

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The antigenic peptide may be flanked by the amino acid residue(s) as defined in (i) and/or (ii) C-terminally, N-terminally or C- and N-terminally.

In one preferred embodiment of the invention the peptide as defined above comprises at least one amino acid sequence of the SEQ ID NO: 185 to 194, 196 to 199, 202 to 205, 207, 209 to 211, 213, 215, 217 to 225, 227 to 234 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 196, 197, 198, 199, 202, 203, 204, 205, 207, 209, 210, 211, 213, 215, 217, 218, 219, 220, 221, 222, 223, 224, 225, 227, 228, 229, 230, 231, 232, 233 and/or 234.

The functional active fragment may be further characterized by structural features. Accordingly, in one preferred embodiment of the invention the functional active fragments consists of at least 60 %, preferably at least 70 %, more preferably at least 80 %, still more preferably at least 90 %, even more preferably at least 95 %, most preferably 99 % of the antigenic peptide of any of the SEQ ID NOS: 1 to 184, more preferably of the antigenic peptide of any of the SEQ ID NOS: 1 to 94 or 110 to 184, still more preferably of the antigenic peptide of any of the SEQ ID NOS: 1 to 94 and most preferably of the antigenic peptide of any of the SEQ ID NOS: 1 to 55. The functional active fragment as defined

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above may be derived from the peptide by one or more amino acid deletions. The deletions may be C-terminally, N-terminally and/or internally.

Another preferred embodiment of the invention relates to a peptide as defined above in the previous embodiments, wherein the antigen is a functional active variant of an antigen of any of the SEQ ID NOS: 1 to 184 and wherein the variant has at least 50 % sequence identity to the antigen of any of the SEQ ID NOS: 1 to 184, preferably of the SEQ ID NOS: 1 to 94 or 110 to 184, more preferably of the SEQ ID NOS: 1 to 55. In a more preferred embodiment the functional active variant has a sequence identity of at least 60 %, preferably at least 70 %, more preferably at least 80 %, still more preferably at least 90 %, even more preferably at least 95 %, most preferably 99 % to the antigen of any of the SEQ ID NOS: 1 to 184, preferably of the SEQ ID NOS: 1 to 94 or 110 to 184, more preferably of the SEQ ID NOS: 1 to 55.

The percentage of sequence identity can be determined e.g. by sequence alignment. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms have been described e.g. in Smith and Waterman, Adv. Appl. Math. 2: 482, 1981 or Pearson and Lipman, Proc. Natl. Acad. Sci. US. A. 85: 2444, 1988.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., J. Mol. Biol. 215: 403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

Variants of an antigen of any of the sequences of SEQ ID NOS: 1 to 184 are typically characterized using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of at least 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment is performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Methods for determining sequence identity over such short windows such as

15 amino acids or less are described at the website that is maintained by the National Center for Biotechnology Information in Bethesda, Maryland.

The functional active variant of an antigen is obtain by sequence alterations in the antigen,
5 wherein the antigen with the sequence alterations retains a function of the unaltered antigen, such as it specifically binds a Men B specific antibody that binds an unaltered form of the antigen. Such sequence alterations can include, but are not limited to, conservative substitutions, deletions, mutations and insertions. Preferably, the functional active variant exhibits reactivity with human sera of convalescing Men B patients, more
10 preferably mediates seroconversion and most preferably shows bactericidal activity. These characteristics of the functional active variant can be assessed e.g. as detailed in Examples 1, 3 and 4. In the context of the present invention a variant specifically binds a Men B specific antibody, exhibits reactivity with human sera of convalescing Men B patients, mediates seroconversion or shows bactericidal activity, if the activity of the variant
15 amounts to at least 10 %, preferably at least 25 %, more preferably at least 50 %, even more preferably at least 70 %, still more preferably at least 80 %, especially at least 90 %, particularly at least 95 %, most preferably at least 99 % of the activity of the antigen without sequence alterations.

20 The term "functional active variant" includes naturally-occurring allelic variants, as well as mutants or any other non-naturally occurring variants. As is known in the art, an allelic variant is an alternate form of a (poly)peptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does essentially not alter the biological function of the polypeptide. By "biological function" is meant a function of the
25 polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. The biological function is distinct from the antigenic function. A polypeptide can have more than one biological function.

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Within any species of the living world, allelic variation is the rule. For example, any bacterial species, e.g. *N. meningitidis*, is usually represented by a variety of strains

(characterized by clonal reproduction) that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfils the same biological function in different strains can have an amino acid sequence that is not identical in each of the strains. Such an allelic variation is equally reflected at the polynucleotide level.

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Allelic variation is very common within the meningitides species. As a matter of example, allelic diversity of the two transferring-binding protein B gene isotypes among a collection of *N. meningitidis* strains representative of serogroup B has been studied by Rokbi B and coworkers (Rokbi B et al., 2000, Infection and Immunity 68: 4938-4947).

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In a preferred embodiment, the functional active variant or fragment derived from the antigen by amino acid exchanges, deletions or insertions may also conserve, or more preferably improve, the activity (reactivity, seroconversion and/or bactericidal activity as defined above). Furthermore, these peptides may also cover epitopes, which trigger the same or preferably an improved T cell response. These epitope are referred to as "heteroclitic". They have a similar or preferably greater affinity to MHC/HLA molecules, and the ability to stimulate the T cell receptors (TCR) directed to the original epitope in a similar or preferably stronger manner. Heteroclitic epitopes can be obtained by rational design i. e. taking into account the contribution of individual residues to binding to MHC/HLA as for instance described by (Rammensee, H. et al., 1999, Immunogenetics. 50: 213-219), combined with a systematic exchange of residues potentially interacting with the TCR and testing the resulting sequences with T cells directed against the original epitope. Such a design is possible for a skilled man in the art without much experimentation.

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In a still more preferred embodiment of the invention the functional active variant of an antigen of any of the SEQ ID NOS: 1 to 184 having at least 50 % sequence identity to the antigen of any of the SEQ ID NOS: 1 to 184, especially at least 60 %, preferably at least 70 %, more preferably at least 80 %, still more preferably at least 90 %, even more preferably at least 95 %, most preferably 99 % to the antigen of any of the SEQ ID NOS: 1 to 184 is derived from the antigen of any of the sequences of SEQ ID NOS: 1 to 184 by conservative substitutions. Conservative substitutions are those that take place within a family of amino acids that are related in their side chains and chemical properties.

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Examples of such families are amino acids with basic side chains, with acidic side chains, with non-polar aliphatic side chains, with non-polar aromatic side chains, with uncharged polar side chains, with small side chains, with large side chains etc.. In one embodiment, one conservative substitution is included in the peptide. In another embodiment, two
 5 conservative substitutions or less are included in the peptide. In a further embodiment, three conservative substitutions or less are included in the peptide.

Examples of conservative amino acid substitutions include, but are not limited to, those listed below:

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<u>Original Residue</u>	<u>Conservative Substitutions</u>
Ala	Ser
Arg	Lys
Asn	Gln; His
15 Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
20 He	Leu, Val
Leu	He; Val
Lys	Arg; Gln; Asn
Met	Leu; He
Phe	Met; Leu; Tyr
25 Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	He; Leu

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In another embodiment of the invention the peptide as defined above may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity

(as defined above for fragments and variants) as the modified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether C-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form an ester, or converted to an amide.

5 Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be converted to an amide. Hydroxyl groups of the peptide side chains may be converted to alkoxy or to an ester using well recognized techniques. Phenyl and phenolic rings of the peptide side chains may be
10 substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with alkyl, alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Thiols can be protected with any one of a number of well recognized protecting groups, such as acetamide groups.

15 Peptides of this invention may be in combination with outer surface proteins or other proteins or antigens of other proteins. In such combination, the antigen may be in the form of a fusion protein. The antigen of the invention may be optionally fused to a selected polypeptide or protein derived from other microorganisms. For example, an antigen or polypeptide of this invention may be fused at its N-terminus or C-terminus to a polypeptide
20 from another pathogen or to more than one polypeptide in sequence. Polypeptides which may be useful for this purpose include polypeptides identified by the prior art.

In a preferred embodiment of the invention the peptide of the invention is fused to an epitope tag which provides an epitope to which an anti-tag substance can selectively bind.

25 The epitope tag is generally placed at the amino- or carboxyl-terminus of the peptide but may be incorporated as an internal insertion or substitution as the biological activity permits. The presence of such epitope-tagged forms of a peptide can be detected using a substance such as an antibody against the tagged peptide. Also, provision of the epitope tag enables the peptide to be readily purified by affinity purification using an anti-tag antibody
30 or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine

(poly-his), poly-histidine-glycine (poly-his-gly) tags, the HA tag polypeptide, the c-myc tag, the Strep tag and the FLAG tag.

Fusions also may include the peptides or antigens of this invention fused or coupled to moieties other than amino acids, including lipids and carbohydrates. Further, antigens of this invention may be employed in combination with other vaccinal agents described by the prior art, as well as with other species of vaccinal agents derived from other microorganisms. Such proteins are useful in the prevention, treatment and diagnosis of diseases caused by a wide spectrum of *Neisseriae* isolates.

These fusion proteins are constructed for use in the methods and compositions of this invention. These fusion proteins or multimeric proteins may be produced recombinantly, or may be synthesized chemically.

The peptides of the invention may be prepared by any of a number of conventional techniques. Desired peptides may be chemically synthesized. An alternative approach involves generating the fragments of known peptides by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes, expressing the digested DNA and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired peptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed as the 5' and 3' primers in the PCR. Techniques for making mutations, such as deletions, insertions and substitutions, at predetermined sites in DNA, and therefore in proteins, having a known sequence are well known. One of skill in the art using conventional techniques, such as PCR, may readily use the antigens and peptides provided herein to identify and isolate other similar proteins. Such methods are routine and not considered to require undue experimentation, given the information provided herein. For example, variations can be made using oligonucleotide-mediated site-directed mutagenesis (Carter et al., *Nucl. Acids Res.*, 13:4331 (1986); Zoller et al., *Nucl. Acids Res.* 10:6487 (1987)), cassette mutagenesis (Wells et al., *Gene*, 34:315 (1985)), restriction selection mutagenesis (Wells et al., *Philos. Trans. R. Soc. London SerA*,

317:415 (1986)), PCR mutagenesis, or other known techniques can be performed on the cloned DNA to produce the peptide of the invention.

Another subject of the present invention relates to a nucleic acid encoding a peptide of the invention, i.e. any peptide as defined above. Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA or cRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA e.g. obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be triple-stranded, double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. Nucleic acid molecule as used herein also refers to, among other, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition, nucleic acid molecule as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA.

The nucleic acid may be a fragment of a nucleic acid occurring naturally in *N. meningitidis*, particularly in *N. meningitidis* B, especially in *N. meningitidis* B strain MC58. The nucleic acid also includes sequences that are a result of the degeneration of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all nucleotide sequences are included in the invention which result in the peptide as defined above.

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Additionally, the nucleic acid may contain one or more modified bases. Such nucleic acids may also contain modifications e.g. in the ribose-phosphate backbone to increase stability and half life of such molecules in physiological environments. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acid molecule" as that feature is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are nucleic acid molecule within the context of the present invention. It will be appreciated that a great

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variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term nucleic acid molecule as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of nucleic acid molecule, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia. For example, nucleotide substitutions can be made which do not affect the polypeptide encoded by the nucleic acid, and thus any nucleic acid molecule which encodes an antigen or fragment or functional active variant thereof as defined above is encompassed by the present invention.

Furthermore, any of the nucleic acid molecules encoding an antigen of the invention or fragment or functional active variant thereof can be functionally linked, using standard techniques such as standard cloning techniques, to any desired regulatory sequences, whether a *N. meningitidis* regulatory sequence or a heterologous regulatory sequence, heterologous leader sequence, heterologous marker sequence or a heterologous coding sequence to create a fusion protein.

The nucleic acid of the invention may be originally formed *in vitro* or in a cell in culture, in general, by the manipulation of nucleic acids by endonucleases and/or exonucleases and/or polymerases and/or ligases and/or recombinases or other methods known to the skilled practitioner to produce the nucleic acids.

In one embodiment of the invention the nucleic acid is located in a vector. A vector may additionally include nucleic acid sequences that permit it to replicate in the host cell, such as an origin of replication, one or more therapeutic genes and/or selectable marker genes and other genetic elements known in the art such as regulatory elements directing transcription, translation and/or secretion of the encoded protein. The vector may be used to transduce, transform or infect a cell, thereby causing the cell to express nucleic acids and/or proteins other than those native to the cell. The vector optionally includes materials to aid in achieving entry of the nucleic acid into the cell, such as a viral particle, liposome, protein coating or the like. Numerous types of appropriate expression vectors are known in the art for protein expression, by standard molecular biology techniques. Such vectors are

selected from among conventional vector types including insects, e.g., baculovirus expression, or yeast, fungal, bacterial or viral expression systems. Other appropriate expression vectors, of which numerous types are known in the art, can also be used for this purpose. Methods for obtaining such expression vectors are well-known (see, e.g. 5 Sambrook et al, Molecular Cloning. A Laboratory Manual, 2d edition, Cold Spring Harbor Laboratory, New York (1989)). In one embodiment, the vector is a viral vector. Viral vectors include, but are not limited to, retroviral and adenoviral vectors.

Suitable host cells or cell lines for transfection by this method include bacterial cells. For 10 example, the various strains of *E. coli* are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, *Streptomyces*, and other bacilli and the like may also be employed in this method. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the peptides of the present invention. Other fungal cells or insect cells such as *Spodoptera frugiperda* (Sf9) 15 cells may also be employed as expression systems. Alternatively, mammalian cells, such as human 293 cells, Chinese hamster ovary cells (CHO), the monkey COS-I cell line or murine 3T3 cells derived from Swiss, BALB/c or NIH mice may be used. Still other suitable host cells, as well as methods for transfection, culture, amplification, screening, production, and purification are known in the art.

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A peptide of the invention may be produced by expressing a nucleic acid of the invention in a suitable host cell. The host cells can be transfected, e.g. by conventional means such as electroporation with at least one expression vector containing a nucleic acid of the invention under the control of a transcriptional regulatory sequence. The transfected or 25 transformed host cell is then cultured under conditions that allow expression of the protein. The expressed protein is recovered, isolated, and optionally purified from the cell (or from the culture medium, if expressed extracellularly) by appropriate means known to one of skill in the art. For example, the proteins are isolated in soluble form following cell lysis, or extracted using known techniques, e.g. in guanidine chloride. If desired, the peptides or 30 fragments of the invention are produced as a fusion protein. Such fusion proteins are those described above. Alternatively, for example, it may be desirable to produce fusion proteins to enhance expression of the protein in a selected host cell or to improve purification. The

molecules comprising the peptides and antigens of this invention may be further purified using any of a variety of conventional methods including, but not limited to: liquid chromatography such as normal or reversed phase, using HPLC, FPLC and the like; affinity chromatography (such as with inorganic ligands or monoclonal antibodies); size
5 exclusion chromatography; immobilized metal chelate chromatography; gel electrophoresis; and the like. One of skill in the art may select the most appropriate isolation and purification techniques without departing from the scope of this invention. Such purification provides the antigen in a form substantially free from other proteinaceous and non-proteinaceous materials of the microorganism.

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Another subject of the invention is a pharmaceutical composition, especially a vaccine, comprising

- at least one peptide according to the invention; and/or
- at least one polypeptide comprising a peptide according to the invention.

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A peptide or polypeptide of the invention may be used for methods for immunizing or treating humans and/or animals with the disease caused by infection with *N. meningitidis*, particularly serogroup B. Therefore, the (poly)peptide may be used within a pharmaceutical composition. The pharmaceutical composition of the present invention may
20 further encompass pharmaceutically acceptable carriers and/or excipients. The pharmaceutically acceptable carriers and/or excipients useful in this invention are conventional and may include buffers, stabilizers, diluents, preservatives, and solubilizers. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical
25 delivery of the (poly)peptides herein disclosed. In general, the nature of the carrier or excipients will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid
30 compositions (e. g. powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical

compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

- 5 In a preferred embodiment the pharmaceutical composition further comprises an immunostimulatory substance such as an adjuvant. The adjuvant can be selected based on the method of administration and may include mineral oil-based adjuvants such as Freund's complete and incomplete adjuvant, Montanide incomplete Seppic adjuvant such as ISA, oil in water emulsion adjuvants such as the Ribi adjuvant system, syntax adjuvant formulation
10 containing muramyl dipeptide, or aluminum salt adjuvants. Preferably, the adjuvant is a mineral oil-based adjuvant, most preferably ISA206 (SEPPIC, Paris, France).

In a more preferred embodiment the immunostimulatory substance is selected from the group comprising polycationic polymers, especially polycationic peptides such as
15 polyarginine, immunostimulatory deoxynucleotides (ODNs), peptides containing at least two LysLeuLys motifs, especially KLKLLLLLKLK, neuroactive compounds, especially human growth hormone, alum, adjuvants or combinations thereof. Preferably the combination is either a polycationic polymer and immunostimulatory deoxynucleotides or of a peptide containing at least two LysLeuLys motifs and immunostimulatory
20 deoxynucleotides. In a still more preferred embodiment the polycationic polymer is a polycationic peptide.

In an even more preferred embodiment of the invention the immunostimulatory substance is at least one immunostimulatory nucleic acid. Immunostimulatory nucleic acids are e.g.
25 neutral or artificial CpG containing nucleic acids, short stretches of nucleic acids derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine dinucleotides (CpG) in a defined base context (e.g. as described in WO 96/02555). Alternatively, also nucleic acids based on inosine and cytidine as e.g. described in WO 01/93903, or deoxynucleic acids containing deoxy-inosine and/or
30 deoxyuridine residues (described in WO 01/93905 and WO 02/095027) may preferably be used as immunostimulatory nucleic acids in the present invention. Preferably, mixtures of different immunostimulatory nucleic acids are used in the present invention. Additionally,

the aforementioned polycationic compounds may be combined with any of the immunostimulatory nucleic acids as aforementioned. Preferably, such combinations are according to the ones described in WO 01/93905, WO 02/32451, WO 01/54720, WO 01/93903, WO 02/13857 and WO 02/095027 and the Australian patent application
5 A 1924/2001.

In addition or alternatively, such vaccine composition may comprise a neuroactive compound. Preferably, the neuroactive compound is human growth factor, e.g. described in WO 01/24822. Also preferably, the neuroactive compound is combined with any of the
10 polycationic compounds and/or immunostimulatory nucleic acids as defined above.

The composition may be used e.g. for immunization or treatment of a subject. The pharmaceutical composition encompasses at least one peptide of the invention; however, it may also contain a cocktail (i.e., a simple mixture) containing different peptides (including
15 fragments and variants) or polypeptides of the invention, optionally mixed with different antigenic proteins or peptides of other pathogens. Such mixtures of these peptides, polypeptides, proteins or fragments or variants thereof are useful e.g. in the generation of desired antibodies to a wide spectrum of Neisseriae isolates. The (poly)peptide(s) of the present invention may also be used in the form of a pharmaceutically acceptable salt.
20 Suitable acids and bases which are capable of forming salts with the peptides of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

Still another subject of the invention is a pharmaceutical composition containing a nucleic
25 acid selected from the group consisting of:

- (a) a nucleic acid of the invention as defined above;
- (b) a nucleic acid coding for the polypeptide comprising a peptide of the invention as defined above; or
- (c) a nucleic acid complementary to the nucleic acid of (a) or (b).

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The nucleic acid sequences, alone or in combination with other nucleic acid sequences encoding antigens or antibodies or directed to other pathogenic microorganisms, may

further be used as components of a pharmaceutical composition. The composition may be used for immunizing or treating humans and/or animals with the disease caused by infection with *N. meningitidis*, particularly serogroup B.

- 5 In another embodiment, the nucleic acid sequences of this invention, alone or in combination with nucleic acid sequences encoding other antigens or antibodies from other pathogenic microorganisms, may further be used in compositions directed to actively induce a protective immune response in a subject to the pathogen. These components of the present invention are useful in methods for inducing a protective immune response in
10 humans and/or animals against infection with *N. meningitidis*, particularly serogroup B.

For use in the preparation of the therapeutic or vaccine compositions, nucleic acid delivery compositions and methods are useful, which are known to those of skill in the art. The nucleic acid of (a), (b) or (c) may be employed in the methods of this invention or in the
15 compositions described herein as DNA sequences, either administered as naked DNA, or associated with a pharmaceutically acceptable carrier and provide for *in vivo* expression of the antigen, peptide or polypeptide. So-called "naked DNA" may be used to express the antigen, peptide or polypeptide of the invention *in vivo* in a patient. (See, e.g., J. Cohen, Science, 259:1691-1692, which describes similar uses of "naked DNA"). For example,
20 "naked DNA" associated with regulatory sequences may be administered therapeutically or as part of the vaccine composition e.g., by injection.

Alternatively, a nucleic acid encoding the antigens or peptides or polypeptides of the invention or a nucleic acid complementary thereto may be used within a pharmaceutical
25 composition, e.g. in order to express the antigens or peptides or polypeptides of the invention *in vivo*, e.g., to induce antibodies.

A preferred embodiment of the invention relates to a pharmaceutical composition, wherein the nucleic acid according to (a), (b) or (c) as defined above is comprised in a vector and/or
30 a cell. Vectors and cells suitable in the context of the present invention are described above. Vectors are particularly employed for a DNA vaccine. An appropriate vector for delivery may be readily selected by one of skill in the art. Exemplary vectors for *in vivo*

gene delivery are readily available from a variety of academic and commercial sources, and include, e.g., adeno-associated virus (International patent application No. PCT/US91/03440), adenovirus vectors (M. Kay et al, Proc. Natl. Acad. Sci. USA, 91:2353 (1994); S. Ishibashi et al, J. Clin. Invest., 92:883 (1993)), or other viral vectors, e.g.,
5 various poxviruses, vaccinia, etc.. Recombinant viral vectors, such as retroviruses or adenoviruses, are preferred for integrating the exogenous DNA into the chromosome of the cell.

Another subject of the invention relates to an antibody or functional active fragment
10 thereof which binds specifically to the antigen of the invention. The present invention includes, for example, monoclonal and polyclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression library.

In a preferred embodiment the antibody is a monoclonal, polyclonal, chimeric or
15 humanized antibody or functional active fragment thereof. In another preferred embodiment the functional active fragment comprises a Fab fragment.

Antibodies generated against the antigens, fragments or variants thereof of the present invention can be obtained by direct injection of the antigens, fragments or variants thereof
20 into an animal or by administering the antigens, fragments or variants thereof to an animal, preferably a non-human. The antibody so obtained will then bind the antigens, fragments or variants. Such antibodies can then be used to isolate reactive antigens, fragments or variants thereof from tissue expressing those.

25 For preparation of monoclonal antibodies, any technique known in the art, which provides antibodies produced by continuous cell line cultures, e.g. a hybridoma cell line, can be used.

Techniques described for the production of single chain antibodies (U. S. Patent No.
30 4,946,778) can be adapted to produce single chain antibodies to the antigens, fragments or variants thereof according to this invention. Also, transgenic mice or other organisms such

as other mammals may be used to express humanized antibodies to antigens, fragments or variants thereof according to this invention.

Still another subject of the invention relates to a hybridoma cell line which produces the
5 antibody of the invention.

Hybridoma cell lines expressing desirable monoclonal antibodies are generated by well-known conventional techniques. The hybridoma cell can be generated by fusing a normal-activated, antibody-producing B cell with a myeloma cell. In the context of the present
10 invention the hybridoma cell is able to produce an antibody specifically binding to the antigen of the invention.

Similarly, desirable high titer antibodies are generated by applying known recombinant techniques to the monoclonal or polyclonal antibodies developed to these antigens (see,
15 e.g., PCT Patent Application No. PCT/GB85/00392; British Patent Application Publication No. GB2188638A; Amit et al., Science, 233:747-753 (1986); Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10033 (1989); PCT Patent Application No. WO90/07861; Riechmann et al., Nature, 332:323-327 (1988); Huse et al., Science, 246:1275-1281 (1988)).

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The present invention also provides a method for producing an antibody according to the invention, characterized by the following steps:

- (a) administering an effective amount of the peptide according to the invention to an animal; and
- 25 (b) isolating the antibody produced by the animal in response to the administration of step (a) from the animal.

Another subject of the invention relates to a method for producing an antibody according to the invention, characterized by the following steps:

- 30 (a) contacting a B cell with an effective amount of the peptide according to the invention;
- (b) fusing the B cell of step (a) with a myeloma cell to obtain a hybridoma cell; and

(c) isolating the antibody produced by the cultivated hybridoma cell.

More particularly, the antibody may be produced by initiating an immune response in a non-human animal by administering a peptide or polypeptide of the invention to an animal, removing an antibody containing body fluid from said animal, and producing the antibody by subjecting said antibody containing body fluid to further purification steps. Alternatively, the antibody may be produced by initiating an immune response in a non-human animal by administering an antigen, fragment or variant thereof, as defined in the present invention, to said animal, removing the spleen or spleen cells from said animal and/or producing hybridoma cells of said spleen or spleen cells, selecting and cloning hybridoma cells specific for said antigen, fragment or variant thereof and producing the antibody by cultivation of said cloned hybridoma cells. In a preferred embodiment the antibody produced according to a method of the invention is additionally purified. Methods of purification are known to the skilled artisan.

The antibody may be used in methods for treating an infection. Accordingly, still another subject of the invention relates to a pharmaceutical composition, especially a vaccine, comprising the antibody of the invention. The pharmaceutical composition may encompass further components as detailed above. The composition may further encompass substances increasing their capacity to stimulate T cells. These include T helper cell epitopes, lipids or liposomes or preferred modifications as described in WO01/78767. Another way to increase the T cell stimulating capacity of epitopes is their formulation with immune stimulating substances for instance cytokines or chemokines like interleukin-2, -7, -12, -18, class I and II interferons (IFN), especially IFN-gamma, GM-CSF, TNF-alpha, flt3-ligand and others.

In another aspect of the invention the peptide, the polypeptide or the nucleic acid of the invention is used for the manufacture of a medicament for the immunization of a subject, preferably against *N. meningitidis*, more preferably against *N. meningitidis* serogroup B.

The peptides, polypeptides or the nucleic acids of the invention are generally useful for inducing an immune response in a subject. The vaccine used for immunization may be

administered to a subject susceptible to infection by *N. meningitidis*, preferably mammals, and still more preferably humans, in any conventional manner, including oral, intranasal, intramuscular, intra-lymph node, intradermal, intraperitoneal, subcutaneous, and combinations thereof, but most preferably through intramuscular injection. The volume of the dose for intramuscular administration is preferably up to about 5 ml, still more preferably between 1 ml and 3 ml, and most preferably about 2 ml. The volume of the dose when intramuscular injection is the selected administration route is preferably up to about 5 ml, still more preferably between 1 ml and 3 ml, and most preferably about 2 ml. The amount of protein comprising the antigen in each dose should be enough to confer effective immunity against and decrease the risk of developing clinical signs resulting from *N. meningitidis* infection to a subject receiving a vaccination therewith. Preferably, the unit dose of protein should be up to about 5 µg protein/kg body weight, more preferably between about 0.2 to 3 µg, still more preferably between about 0.3 to 1.5 µg, more preferably between about 0.4 to 0.8 µg, and still more preferably about 0.6 µg. Alternative preferred unit doses of protein could be up to about 6 µg protein/kg body weight, more preferably between about 0.05 to 5 µg, still more preferably between about 0.1 to 4 µg. The dose is preferably administered 1 to 3 times, e.g. with an interval of 1 to 3 weeks. Preferred amounts of protein per dose are from approximately 1 µg to approximately 1 mg, more preferably from approximately 5 µg to approximately 500 µg, still more preferably from approximately 10 µg to approximately 250 µg and most preferably from approximately 25 µg to approximately 100 µg.

In still another aspect of the invention the antibody of the invention or functional fragment thereof is used for the manufacture of a medicament for the treatment of an infection, preferably a *N. meningitidis* infection, more preferably a *N. meningitidis* serogroup B infection. The treatment involves administering an effective amount of an antibody of the invention to a subject, preferably a mammal, more preferably a human. Thus, antibodies against the antigens, fragments or variants thereof of the present invention may be employed to inhibit and/or treat infections, particularly bacterial infections and especially infections arising from *N. meningitidis*, especially serogroup B.

An "effective amount" of a nucleic acid composition may be calculated as that amount capable of exhibiting an *in vivo* effect, e.g. preventing or ameliorating a sign or symptom of infection, particularly *N. meningitidis* infection, especially of serogroup B. Such amounts may be determined by one of skill in the art. Preferably, such a composition is administered parenterally, preferably intramuscularly or subcutaneously. However, it may also be formulated to be administered by any other suitable route, including orally or topically. The selection of the route of delivery and dosage of such therapeutic compositions is within the skill of the art.

10 Treatment in the context of the present invention refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

15

Another subject of the invention relates to a method of diagnosing a *N. meningitidis* infection comprising the steps of:

- (a) contacting a sample obtained from a subject with the peptide according to the invention; and
- 20 (b) detecting the presence of an antibody against *N. meningitidis* in the sample.

The peptides or polypeptides of the invention may be used for the detection of the *N. meningitidis*, particularly serogroup B. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease, most preferably for the diagnosis of a *N. meningitidis* infection. The peptides or polypeptides may be used to detect the presence of a *N. meningitidis*-specific antibody or fragment thereof e.g. in a sample obtained from a subject. The sample may be e.g. a blood sample. Alternatively, the presence of a *N. meningitidis*-specific antigen can be detected using an antibody of the invention.

30 Accordingly, an alternative method of diagnosing a *N. meningitidis* infection comprises the steps of:

- (a) contacting a sample obtained from a subject with the antibody according to the invention; and
- (b) detecting the presence of an antigen of *N. meningitidis* in the sample.

5 The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of the peptides or antibodies of the present invention in cells and tissues or body fluids, including determination of normal and abnormal levels. Assay techniques that can be used to determine levels of a peptide or an antibody, in a sample derived from a host are well known to those of skill in the art. Such assay methods include
10 radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these, ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to the peptide, particularly the antigen, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as
15 radioactive, fluorescent or enzymatic reagent, such as horseradish peroxidase enzyme.

The peptides or antibodies of the present invention may also be used for the purpose of or in connection with an array. More particularly, at least one of the peptides or antibodies of the present invention may be immobilized on a support. Said support typically comprises a
20 variety of antigens and fragments thereof whereby the variety may be created by using one or several of the peptides or antibodies of the present invention. The characterizing feature of such array as well as of any array in general is the fact that at a distinct or predefined region or position on said support or a surface thereof, a distinct polypeptide is immobilized. Because of this any activity at a distinct position or region of an array can be
25 correlated with a specific polypeptide. The number of different peptides or antibodies of the present invention immobilized on a support may range from as little as 10 to several 1000 different peptides or antibodies of the present invention.

The manufacture of such arrays is known to the one skilled in the art and, for example,
30 described in US patent 5,744,309. The array preferably comprises a planar, porous or non-porous solid support having at least a first surface. Preferred support materials are, among others, glass or cellulose. It is also within the present invention that the array is used for

any of the diagnostic applications described herein. Apart from the peptides or antibodies of the present invention also the nucleic acid molecules according to the present invention may be used for the generation of an array as described above.

5 Another aspect of the invention relates to a method for identifying a ligand capable of binding to a peptide according to the invention comprising:

- (a) providing a test system comprising the peptide,
 - (b) contacting the test system with a test compound, and
 - (c) detecting a signal generated in response to the binding of the test compound to the
- 10 peptide.

More particularly, the method may be carried out by contacting an isolated or immobilized peptide according to the invention with a candidate ligand under conditions to permit binding of the candidate ligand to the peptide, wherein the test system comprises a

15 component capable of providing a detectable signal in response to the binding of the candidate ligand to said peptide; and detecting the presence or absence of a signal generated in response to the binding of the ligand to the peptide. The ligand may be a agonist or an antagonist.

20 Test systems for detection binding of a ligand are known to the skilled artisan and include e.g. binding assays with labeled ligand such as radioligands, fluorescence-labeled ligands or enzyme-labeled ligands.

The test compound can be any test compound either naturally occurring or chemically

25 synthesized. Naturally occurring test compounds include in particular antibodies, preferably those showing similarity to the antibodies of the invention. In one preferred embodiment of the invention the test compound is provided in the form of a chemical compound library. Chemical compound libraries include a plurality of chemical compounds and have been assembled from any of multiple sources, including chemically

30 synthesized molecules and natural products, or have been generated by combinatorial chemistry techniques. They are especially suitable for high throughput screening. They

may be comprised of chemical compounds of a particular structure or compounds of a particular creature such as a plant.

The method for identifying a ligand may also include the following steps:

- 5 (a) providing a peptide according to the invention,
- (b) providing an interaction partner to the peptide, especially an antibody according to the invention,
- (c) allowing interaction of the peptide to said interaction partner to form a interaction complex,
- 10 (d) providing a test compound,
- (e) allowing a competition reaction to occur between the test compound and the interaction complex, and
- (f) determining whether the test compound inhibits or reduces the interaction activities of the peptide with the interaction partner.

15

The ligands identified may be employed, for instance, to inhibit diseases arising from infection with *Neisseria*, especially *N. meningitidis* and may therefore be formulated in a pharmaceutical composition.

- 20 In a last aspect, the peptide according to the invention is used for the isolation and/or purification and/or identification of a ligand of the peptide, wherein the isolation and/or purification and/or identification of the ligand may be carried out as detailed above or as known to the person skilled in the art. In a preferred embodiment of the invention an affinity device may be used. The affinity device may comprise as least a support material
- 25 and any peptide according to the present invention, which is attached to the support material. Because of the specificity of the peptides according to the present invention for their target cells or target molecules or their interaction partners, the peptides allow a selective removal of their interaction partner(s) from any kind of sample applied to the support material provided that the conditions for binding are met. The sample may be a
- 30 biological or medical sample, including but not limited to, fermentation broth, cell debris, cell preparation, tissue preparation, organ preparation, blood, urine, lymph liquid, liquor and the like. The peptide may be attached to the matrix in a covalent or non-covalent

manner. Suitable support material is known to the one skilled in the art and can be selected from the group comprising cellulose, silicon, glass, aluminium, paramagnetic beads, starch and dextrane.

5 The present invention is further illustrated by the following figures, examples and the sequence listing, from which further features, embodiments and advantages may be taken. It is to be understood that the present examples are given by way of illustration only and not by way of limitation of the disclosure.

10 Figure 1 shows the measurement of anti-meningococcal antibody levels and the determination of seroconversion, shows immunoblotting with human sera to detect antigenic proteins of *Neisseria meningitides* and determination of serum bactericidal activity (SBA) of human sera.

15 Figure 2 shows the generation of genomic libraries.

Figure 3 shows the seroconversion of peptides tested with patient sera.

20 Figures 4 and 5 show the detection of epitope-specific antibodies in sera of *E. coli* lysate immunized mice.

Figure 6 shows the Western blot analysis for detection of MenB proteins.

25 Figure 7 shows the bactericidal activity of mouse sera.

Table 1 shows the list of serum samples obtained for antigen characterization.

30 Table 2 shows the summary for the characterization of selected serum samples.

Table 3 shows the antigens identified by bacterial surface display screens.

Table 4 shows characteristics of MenB peptides.

Table 5 summarizes characteristics of synthetic peptides and the respective clones

- 5 The figures and tables to which it might be referred to in the specification are described in the following in more detail.

FIGURES

10

Figure 1 A shows the measurement of anti-meningococcal antibody levels and determination of seroconversion. ELISA plates were coated with 10 µg/ml total protein present in bacterial lysates prepared from *Neisseria meningitidis* serogroup **B**. Standard ELISA was performed with different serum dilutions and bound IgG was detected with
15 anti-human IgG secondary antibody. A representative experiment is shown at 10,000 X serum dilution, comparing IgG reactivities of convalescent and acute phase sera.

Figure 1 B shows the immunoblotting with human sera to detect antigenic proteins of *Nisseria meningitidis*. Total bacterial lysate was prepared by a freeze-thaw method,
20 proteins separated by SDS-PAGE and transferred to nitrocellulose membrane which was probed with human serum samples at a 5,000 X dilution and mouse anti-human IgG as a secondary antibody.

**Figure 1 C shows the determination of serum bactericidal activity (SBA) of human
25 sera.** Bactericidal titers of pre-selected sera were determined in a colorimetric assay, using heat-inactivated human sera in the indicated dilutions, guinea pig serum as complement source and the MenB MC58 strain.

Figure 2 shows genomic libraries. Size distribution of 387 randomly picked clones of
30 NMB300 and of 409 randomly picked clones of NMB50. Numbers in brackets indicate the expected and the actual, average insert size.

Figure 3 shows seroconversion of peptides tested with patient sera. Seroconversion data of 2 exemplary peptides and of MenB lysate are shown for 7 serum pairs from patients. Whereas the bacterial lysate (bottom) and peptide NMB 1523.5 (top; +++) show seroconversion, NMB 2061.2 (middle; -) is negative in this test.

5

Figures 4 and 5 show detection of epitope-specific antibodies in sera of *E. coli* lysate immunized mice. **Fig. 4** Western blot analysis was performed on lysates that were prepared from *E. coli* clones selected in BSD screens and used for immunization of mice to generate MenB antigen epitope specific antibodies. The presence of specific antibodies was noticed by the immunodetection of the epitope-carrying platform proteins, LamB or FhuA. **Fig. 5** Peptide ELISA was performed with synthetic peptides representing displayed antigenic epitopes. NMB numbers represent animal sera (.1 to .5; 5 individual mice) and NMB numbers prior to data columns represent peptides as listed in Table 3. LamB and FhuA sera represent negative controls, induced by empty platform protein expressing *E. coli*.

10

Figure 6 shows Western blot analysis for detection of MenB proteins with epitope-induced mouse sera. Total bacterial lysate was prepared from the MC58 strain and proteins were analysed by Western blotting with 5,000 X diluted mouse sera generated with immunization of epitope carrying *E. coli* clones.

15

Figure 7 shows the bactericidal activity of mouse sera containing MenB epitope specific antibodies. Sera that induced >20 % bactericidal activity were considered positive, and were quantified based on the percentage of reduction in bacterial growth. Sera were classified as having pronounced bactericidal activity (>40 %), intermediate (30 - 40 %) or low (20 - 30 %) activity, respectively. Importantly, the bactericidal activity was IgG concentration dependent and the assay was highly reproducible.

20

Table 1: Serum samples for antigen characterization.

25

Serum samples were obtained from 215 donors. Column 3 referred to as "pathogen", indicates which pathogen was identified inducing disease. Column 4 shows the number of

30

paired sera obtained from the same donor in acute and convalescent phase. N.a., not available.

Table 2: Summary of immune characterization of selected serum samples.

5 Sera were analysed for serum bactericidal activity (SBA) and seroconversion (SC). SBA activity is given as highest dilution of serum with bactericidal activity. ELISA U, specifies reactivities of sera with MenB lysate. Seroconversion is given as fold increase comparing acute and convalescent phase sera. SE2A, SE2B, SE2C; bactericidal activity of sera against MenA, B and C, respectively, expressed as highest dilution of serum with positive
10 reaction. The serum numbers labeled by a * refer to the sera that were also used for peptide ELISA as summarized in Table 3.

Table 3: Immunogenicity of epitopes in peptide ELISA - seroconversion.

Peptides derived from MenB antigens were analysed with human sera for immunogenicity
15 and seroconversion by peptide ELISA using biotinylated peptides. The peptide name entails the NMB designation according to the annotation of the MC58 MenB strain. The sequences and the SEQ ID NOS are listed.

Table 4: characteristics of MenB peptides.

20 The table lists all bacterial clones which were injected into mice for the generation of antibodies, including their amino acid sequence and the peptides which were synthesized according to the identified immunogenic epitope. All bacterial clones relating to one ORF (first column) were injected simultaneously. Please note that different BA experiments are separated by a horizontal line. Data obtained with the such generated sera (clone WB -
25 Western blot with *E. coli* clone, WB - Western blot with MenB grown in RPMI (1) or BHI (2), BA - bactericidal activity) therefore relate to single or multiple clones. Data obtained for peptides relate always to the individual peptide (peptide ELISA, SC - seroconversion).

Table 5: Summary of the characteristics of synthetic peptides and the respective clones.

The fragments of the SEQ ID NOS: 1 to 184 were classified into 9 groups (groups I to IX)
5 taking their bactericidal activity data and the seroconversion data of the respective clone(s)
into account.

EXAMPLES

Example 1: Selection of serum sources for the identification and characterization of MenB antigens

5

Experimental procedures

Preparation of total bacterial lysates.

Neisseria meningitidis serogroup B MC58 strain was grown in BHI medium. Overnight
10 cultures were diluted in BHI 1:50 and further grown until an OD_{600 nm} of approximately 0.6 for logarithmic phase and up to OD_{600 nm} of approximately 2.5 for stationary phase cells. Bacteria were harvested by centrifugation, re-suspended in PBS, and the bacterial suspension was freeze-thawed (incubation at -80°C followed by exposure to room temperature) and sonicated for 2 min. Samples were centrifuged and the supernatant
15 fraction recovered and stored in aliquotes at -80°C. Lysate preparations were used for coating ELISA plates (10 µg/ml), for Western blot analysis (10-20 µg total protein), as well as for the induction of hyperimmune mouse sera (~ 300 µg).

Enzyme-linked immunosorbent assay (ELISA).

20 ELISA plates (Maxisorb, Millipore) were coated with 5-10 µg/ml total protein diluted in coating buffer (0.1 M sodium carbonate pH 9.2). Three dilutions of sera (2,000 X, 10,000 X, 50,000 X) were made in PBS-BSA. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000 X). Antigen-antibody complexes
25 were quantified by measuring the conversion of the substrate (ABTS) to colored product based on OD_{405 nm} readings by automatic ELISA reader (TECAN SUNRISE).

Western Blot analysis.

Bacterial cells were grown overnight and were collected by centrifugation and re-
30 suspended in SDS-sample buffer for crude, whole cell extracts. Approximately 10 to 20 µg of total cellular protein was separated by 10% SDS-PAGE and blotted onto HybondC membrane (Amersham Pharmacia Biotech, England) using a semi-dry transfer system

(Bio-Rad) and visualized by Ponceau S staining. After blocking over night in 5% milk, human sera or mouse immune sera were added at 5,000-10,000x dilution, and HRP-labelled goat anti-mouse IgG at a dilution of 1:5,000 (Southern Biotech) were used for specific detection. Detection was performed using the ECL detection kit (Amersham Pharmacia Biotech, England).

In vitro bactericidal assay.

The colorimetric bactericidal assay was performed according to Rodrigez et al. (Clin Diagn Lab Immunol. 9: 109-14, 2002) with minor modifications. *Neisseria meningitidis* serogroup B strain MC58 was grown on agar plates prepared with Mueller-Hinton broth supplemented with 10% fetal bovine serum (MHB-FBS, Sigma Cat.Nr.: M9677) over night at 37°C/5% CO₂. A single colony was picked and growth repeated again. Bacteria were harvested by scraping the agar plates, washed in PBS and diluted in HBSS-B (Hanks' balanced salt solution with 0.1% BSA) to a concentration of 800 CFU per 100 µl based on OD_{600nm} readings of bacterial suspensions. (The actual CFU number was also determined by plating.) Serial serum dilutions were also prepared in HBSS-B. 12.5 µl/well of each the bacterial suspension, the diluted serum samples and the guinea pig-complement (Sigma Cat.Nr.: S1639) were added to 96-well flat-bottom tissue culture-treated plates and volume was adjusted to 50 µl with HBSS-B. For validation purposes, purified IgGs diluted in HBSS-B were used instead of serum (10 and 20 µg). Plates were incubated for 30 min at 37°C without CO₂ before the addition of 150 µl of 0.02% bromocresole purple (Sigma Cat.Nr.: 11,437-25) in Mueller Hinton medium with 2% Glucose. Color change (due to bacterial growth) was detected after 20 h growth at 37°C/5% CO₂ and quantified by spectrophotometry at 405 nm. The percentage of killing was calculated relative to negative and positive controls. The positive control, hyperimmune mouse serum (generated with immunization of mice with total MenB lysate), was set as 100% killing (no bacterial growth) and the negative control (blank) was provided by samples without antibody source (0% killing).

Results

The antibodies induced by pathogens and present in serum and other body fluids constitute a molecular imprint of the *in vivo* expression of the corresponding proteins comprising antigens and have therefore been chosen in our approach as tools for the identification of a comprehensive set of antigens.

Serum samples from individuals (children and adults) diseased by or exposed to *Neisseria meningitidis* were collected as relevant sources of antibodies for antigen identification. The diagnosis of meningococcal disease was based on the isolation of the pathogen from the patients' blood and serogrouping was performed according to routine clinical microbiological methods. From the majority of patients with meningitis two samples were obtained, the first in the acute phase (at the time of admission to the hospital) and the second 2 weeks later during convalescence. Single serum samples from patients were also obtained during recovery. Sera were also collected from contact persons (household members) exposed to the pathogen that diseased their family member. At the time of sampling, nasopharyngeal carriage of *Neisseria meningitidis* was also determined. The 290 serum samples were collected from 215 different donors and stored at -80°C until analysis (summarized in Table 1).

In order to select the most suitable serum samples for identification and characterization of antigens, three different immune assays were performed. First, total anti-meningococcal IgG and IgA levels were determined by ELISA using bacterial lysates prepared from *Neisseria meningitidis* serogroups B and C. In the case of the paired (acute and convalescent) samples, not only the ELISA reactivity served as a basis for positive selection, but also the extent of seroconversion (ratio of convalescent/acute reactivities) (Fig. 1 A). 58 of the 75 patients with paired samples seroconverted (>2 fold increase in antibodies in convalescent sera relative to the acute), and seroconversion rates ranged between 2 and 100 fold.

Since capsular polysaccharides are potent antigens, and dominate the antibody response, it was necessary to ensure that ELISA reactivities of high titer sera originated also from

proteinaceous antigens. For that reason, immunoblotting was performed with pre-selected sera using total bacterial lysate prepared from the MenB MC58 strain. We found a good correlation between the ELISA results and the extent (number of bands) and intensity (strength of signal) of immune reactivity on immunoblots (Fig. 1 B).

5

The highly important function of the antibodies against *Meningococci* is complement-dependent bactericidal killing. To measure the bactericidal titer of the sera, an *in vitro* assay was performed using *Neisseria meningitidis* serogroup B, human serum samples and complement from guinea pig serum. Human serum samples were tested in the 8 to 512-
10 fold dilution range (Fig. 1 C). All of the acute phase sera and some of the convalescent sera were excluded, since antibiotic treatment of the patient donors precluded the analysis. In order to exclude false positive results coming from antibiotics present in the serum, all samples with high killing activity were also tested in the absence of complement. Samples with positive SBA (serum bactericidal activity) were tested repeatedly in two additional
15 experiments.

The convalescent patient sera showed the highest ELISA and killing activity, and only few exposed (but not diseased) individuals had measurable SBA or ELISA reactivity. There was no absolute direct correlation between ELISA and SBA in any of the donor groups.
20 Highest ELISA titers did not always correlate with high killing activity and *vice versa*. The detailed characterization of the sera based on ELISA reactivity, seroconversion and bactericidal activity provided the necessary reagents for antigen identification and characterization.

25 As the result of this comprehensive analysis, 31 sera were selected out of the 290 and pooled into 6 pools (Table 2). Four pools were made with sera from convalescent phase patients with meningitis caused by *Neisseria meningitidis* serogroup B or C (two from each). Two additional pools were prepared from exposed adults, one from colonized, another from non-colonized individuals. The serum pools were depleted of anti-*E. coli*
30 antibodies in order to eliminate background selection in the bacterial surface display screens. After depletion, IgGs were purified by Protein G affinity chromatography. Quality and high purity of IgG preparations were tested by SDS-PAGE.

In summary, the sera that were collected and the IgG pools prepared from these sera were well characterized and provided the necessary reagents to initiate screening of the display libraries based on ELISA reactivity, seroconversion and bactericidal activity.

5

Example 2: Identification of antigenic proteins from *Neisseria meningitidis* serogroup B

10 ***Experimental procedures***

Preparation of genomic DNA.

50 ml culture medium (BHI) were inoculated with *Neisseria meningitidis* B MC58 bacteria from a frozen stab and grown with aeration and shaking for 18 h at 37°C. The culture was then harvested, centrifuged with 1,600 x g for 15 min and the supernatant was removed. Bacterial pellets were washed 3 x with PBS. Genomic DNA was prepared using the Wizard Genomic DNA Purification Kit for GRAM-negative bacteria from Promega according to the instructions of the manufacturer. After the final precipitation with ethanol, DNA was recovered by centrifuging the precipitates with 10,000-12,000 x g, then dried on air and dissolved in ddH₂O.

20

Preparation of small genomic DNA fragments.

Genomic DNA fragments were mechanically sheared into fragments ranging in size between 150 and 300 bp using a cup-horn sonicator (Bandelin Sonoplus UV 2200 sonicator equipped with a BB5 cup horn, 10 sec. pulses at 100 % power output) or into fragments of size between 50 and 70 bp by mild DNase I treatment (Novagen). Sonication yielded a tight fragment size distribution when breaking the DNA into fragments of the 150-300 bp size range. Fragments of 50 to 70 bp in size were obtained by mild DNase I treatment using Novagen's shotgun cleavage kit. A 1:450 dilution of DNase I provided with the kit was prepared and the digestion was performed in the presence of MnCb in a 10 µl volume at 20°C for 10 min to ensure double-stranded cleavage by the enzyme. Reactions were stopped with 2 µl of "Stop-Buffer" (100 mM EDTA, 30 % glycerol, 0.5 %

30

Orange G, 0.075 % xylene cyanol) and the fragmentation efficiency was evaluated on a 2 % TAE-agarose gel. This treatment resulted in total fragmentation of genomic DNA into near 50-70 bp fragments. Fragments were then blunt-ended twice using T4 DNA Polymerase in the presence of 100 µM each of dNTPs to ensure efficient flushing of the
5 ends. Fragments were used immediately in ligation reactions or frozen at -20°C for subsequent use.

Description of the vectors.

The vector pMAL4.31 was constructed on a pASK-IBA backbone with the beta-lactamase
10 {*bid*) gene exchanged with the Kanamycin resistance gene. In addition the *bla* gene was cloned into the multiple cloning site. The sequence encoding mature beta-lactamase is preceded by the leader peptide sequence of *ompA* to allow efficient secretion across the cytoplasmic membrane. Furthermore, a sequence encoding the first 12 amino acids (spacer
15 sequence) of mature beta-lactamase follows the *ompA* leader peptide sequence to avoid fusion of sequences immediately after the leader peptidase cleavage site, since e.g. clusters of positive charged amino acids in this region would decrease or abolish translocation across the cytoplasmic membrane. A *SmaI* restriction site serves for library insertion. An upstream *FseI* site and a downstream *NotI* site, which were used for recovery of the
20 selected fragment, flank the *SmaI* site. The three restriction sites are inserted after the sequence encoding the 12 amino acid spacer sequence in such a way that the *bla* gene is transcribed in the -1 reading frame resulting in a stop codon 15 bp after the *NotI* site. A +1 bp insertion restores the *bla* ORF so that beta-lactamase protein is produced with a consequent gain of Ampicillin resistance.

25 The vector pMAL9.1 was constructed by cloning the *lamB* gene into the multiple cloning site of pEHL. Subsequently, a sequence was inserted in *lamB* after amino acid 154, containing the restriction sites *FseI*, *SmaI* and *NotI*. The reading frame for this insertion was constructed in such a way that transfer of frame-selected DNA fragments excised by digestion with *FseI* and *NotI* from plasmid pMAL4.3 1 yields a continuous reading frame
30 of *lamB* and the respective insert.

The vector pHIE1 1 was constructed by cloning the *fliuA* gene into the multiple cloning site of pEHL. Thereafter, a sequence was inserted *mfliuA* after amino acid 405, containing the restriction site *FseI*, *XbaI* and *NotI*. The reading frame for this insertion was chosen in a way that transfer of frame-selected DNA fragments excised by digestion with *FseI* and
5 *NotI* from plasmid pMAL4.3 1 yields a continuous reading frame of *fhuA* and the respective insert.

Cloning and evaluation of the library for frame selection.

Genomic *N. meningitidis* MC58 DNA fragments were ligated into the *SmaI* site of the
10 vector pMAL4.31. Recombinant DNA was electroporated into DH10B electrocompetent *E. coli* cells (GIBCO BRL) and transformants plated on LB-agar supplemented with Kanamycin (50 µg/ml) and Ampicillin (50 µg/ml). Plates were incubated over night at 37°C and colonies collected for large scale DNA extraction. A representative plate was stored and saved for collecting colonies for colony PCR analysis and large-scale
15 sequencing. A simple colony PCR assay was used to initially determine the rough fragment size distribution as well as insertion efficiency. From sequencing data the precise fragment size was evaluated, junction intactness at the insertion site as well as the frame selection accuracy ($3n+1$ rule).

20 *Cloning and evaluation of the library for bacterial surface display.*

Genomic DNA fragments were excised from the pMAL4.31 vector, containing the *N. meningitidis* MC58 library with the restriction enzymes *FseI* and *NotI*. The entire population of fragments was then transferred into plasmids pMAL9.1 (LamB) or pHIE1 1 (FhuA), which have been digested with *FseI* and *NotI*. Using these two restriction
25 enzymes, which recognise an 8 bp GC rich sequence, the reading frame that was selected in the pMAL4.31 vector is maintained in each of the platform vectors. The plasmid library was then transformed into *E. coli* DH5alpha cells by electroporation. Cells were plated onto large LB-agar plates supplemented with 50 µg/ml of Kanamycin and grown over night at 37°C at a density yielding clearly visible single colonies. Cells were then scraped
30 off the surface of these plates, washed with fresh LB medium and stored in aliquots for library screening at -80°C.

Bacterial surface display screens using MACS (magnetic cell sorting).

Approximately 2.5×10^8 cells from a given library were grown in 5 ml LB-medium supplemented with 50 µg/ml Kanamycin for 2 h at 37°C. Expression was induced by the addition of 1 mM IPTG for 30 min. Cells were washed twice with fresh LB medium and
5 approximately 2×10^7 cells re-suspended in 100 µl LB medium and transferred to an Eppendorf tube.

10 to 20 µg of biotinylated, human IgGs purified from serum was added to the cells and the suspension incubated overnight at 4°C with gentle shaking. 900 µl of LB medium was
10 added, the suspension mixed and subsequently centrifuged for 10 min at 6,000 rpm at 4°C (For IgA screens, 10 µg of purified IgAs were used and these captured with biotinylated anti-human-IgG secondary antibodies). Cells were washed once with 1 ml LB and then re-suspended in 100 µl LB medium. 10 µl of MACS microbeads coupled to streptavidin (Miltenyi Biotech, Germany) were added and the incubation continued for 20 min at 4°C.
15 Thereafter, 900 µl of LB medium was added and the MACS microbead cell suspension was loaded onto the equilibrated MS column (Miltenyi Biotech, Germany) which was fixed to the magnet. (The MS columns were equilibrated by washing once with 1 ml 70% EtOH and twice with 2 ml LB medium.)

20 The column was then washed three times with 3 ml LB medium. After removal of the magnet, cells were eluted by washing with 2 ml LB medium. After washing the column with 3 ml LB medium, the 2 ml eluate was loaded a second time on the same column and the washing and elution process repeated. The loading, washing and elution process was performed a third time, resulting in a final eluate of 2 ml.

25

A second and third round of screening was performed as follows. The cells from the final eluate were collected by centrifugation and re-suspended in 1 ml LB medium supplemented with 50 µg/ml Kanamycin. The culture was incubated at 37°C for 90 min and then induced with 1 mM IPTG for 30 min. Cells were subsequently collected, washed
30 once with 1 ml LB medium and suspended in 10 µl LB medium. 10 to 20 µg of human, biotinylated IgGs were added again and the suspension incubated over night at 4°C with gentle shaking. All further steps were exactly the same as in the first selection round. Cells

selected after two rounds of selection were plated onto LB-agar plates supplemented with 50 µg/ml Kanamycin and grown over night at 37°C.

Evaluation of selected clones by sequencing and Western blot analysis.

- 5 Selected clones were grown overnight at 37°C in 3 ml LB medium supplemented with 50 µg/ml Kanamycin to prepare plasmid DNA using standard procedures. Sequencing was performed at MWG (Germany).

- For Western blot analysis approximately 10 to 20 µg of total cellular protein was separated
10 by 10% SDS-PAGE and blotted onto HybondC membrane (Amersham Pharmacia Biotech, England). The LamB or FhuA fusion proteins were detected using human serum as the primary antibody at a dilution of approximately 1:3,000 to 1:5,000 and anti-human IgG or IgA antibodies coupled to HRP at a dilution of 1:5,000 as secondary antibodies. Detection was performed using the ECL detection kit (Amersham Pharmacia Biotech, England).
15 Alternatively, rabbit anti-FhuA or rabbit anti-LamB polyclonal immune sera were used as primary antibodies in combination with the respective secondary antibodies coupled to HRP for the detection of the fusion proteins.

Results

20

Genomic libraries

- Two genomic libraries (NMB 50 and NMB 300), which contained most, if not all, possible antigenic epitopes encoded by the MenB genome, were generated by randomly fragmenting the genomic DNA of *N. meningitidis* B (MC58) and cloning fragments of an
25 approximate size of 50 and 300 bp (Fig. 2) into vector pMAL4.31. For the two resulting libraries NMB50 (50 bp) and NMB300 (300 bp) a total number of clones after frame selection of at least 6×10^5 and 1.2×10^5 was obtained, respectively.

- Quality of the libraries was assessed by randomly choosing approximately 500 clones and
30 sequencing the same. The bioinformatic analysis showed that the actual insert sizes were close to the intended ones (NMB50: 83bp; NMB300: 215bp) and that the majority of

clones fell within a narrow size range (Fig. 2). It was also shown that the clones were randomly distributed over the *N. meningitidis* B genome.

The display of the MenB peptides on the surface of *E. coli* required transfer of the inserts
5 from the frame selection vector pMAL4.31 to the display plasmids pMAL9.1 (LamB) or
pHIE1 1 (FhuA). MenB genome-derived DNA fragments were excised by *FseI* and *NoI*
restriction enzymes. Ligation of inserts with the respective plasmid DNA resulted in
approximately 5×10^5 individual clones. The *E. coli* clones were scraped off the LB plates
and frozen without further amplification. Successful transfer of the libraries was proven by
10 colony PCR analysis of 24 randomly picked clones from both libraries. Almost all clones
contained an insert of the expected size.

Bacterial surface display screens

The genomic libraries expressing MenB peptide sequences embedded in extracellular loops
15 of LamB and FhuA outer membrane proteins were subjected to MACS-based
immunopanning with the biotinylated human IgG preparations described above
(Example 1, Table 2).

The antigen identification screens have discovered numerous immunogenic peptide
20 sequences (i.e. 184 antigens as defined in Table 3) of *Neisseria meningitidis* serogroup B.
The antigens were selected from 12 different bacterial surface display screens with 6
different IgG screening reagents prepared from pooled sera and two different display
libraries (LamB and FhuA). All of the antigens and those peptides derived from the
respective sequences depicted in Tables 3 to 5 have been confirmed either by Western
25 blotting or by FACS analysis of selected *E. coli* clones. The list of antigens identified with
human sera and genomic libraries displaying MenB peptides provides the basis for the
selection of candidate vaccine antigens for prevention or treatment of meningococcal
infections.

Example 3: Epitope serology of synthetic peptides derived from MenB antigens with human sera

Experimental procedure

5

Peptide ELISA.

Biotin-labeled peptides were coated on Streptavidin ELISA plates (EXICON) at 10 µg/ml concentration according to the manufacturer's instructions. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000 X). Sera were tested at two serum dilutions, 1:400 and 1:2,000. Following manual coating, peptide plates were processed and analyzed by the Gemini 160 ELISA robot (TECAN) with a built-in ELISA reader (GENIOS, TECAN).

15 *Peptide synthesis.*

Peptides were synthesized in small scale (4 mg resin; up to 288 in parallel) using standard F-moc chemistry on a Rink amide resin (PepChem, Tubingen, Germany) using a SyroII synthesizer (MultisynTech, Witten, Germany). After the sequence was assembled, peptides were elongated with Fmoc-epsilon-aminohexanoic acid (as a linker) and biotin (Sigma, St. Louis, MO; activated like a normal amino acid). Peptides were cleaved off the resin with 93% TFA, 5% triethylsilane, and 2% water for one hour. Peptides were dried under vacuum and freeze dried three times from acetonitrile/water (1:1). The presence of the correct mass was verified by mass spectrometry on a Reflex III MALDI-TOF (Bruker, Bremen Germany). The peptides were used without further purification.

25

Results

The identified antigens were further characterized in order to select the most promising vaccine candidates for further animal studies. The validation steps included epitope serology with synthetic peptides and human sera (Example 3) and *in vitro* bactericidal activity with epitope induced hyperimmune mouse sera (Example 4).

30

In order to assess the immunogenicity of identified epitopes, we analysed human serum samples from infected and exposed individuals for the presence of epitope-specific antibodies. Based on the bioinformatic analysis of DNA sequences derived from bacterial clones selected by the encoding immunogenic epitopes, corresponding peptides were designed and synthesized. The peptides (i.e. antigens) are listed in Table 3. In case of epitopes with more than 26 amino acid residues, overlapping peptides were made. All peptides were synthesized with an N-terminal biotin-tag and used as coating reagents on Streptavidin-coated ELISA plates. Epitope serology was performed in two steps. First, peptides were analysed with 21 serum samples that were also used for antigen identification (labeled by an * in Table 2, column "serum-no."). 14 samples were from convalescing MenB, 4 from convalescing MenC infected patients, and 3 from exposed, but not colonized family members. Peptide-specific antibodies were detected by standard ELISA at two different serum dilutions (400x and 2,000x). Extent of reactivity was calculated based on median ELISA units of the 21 serum samples. Immune reactivity of peptides ranged from highly and widely reactive to weakly positive or negative. The results are summarized in Table 3 (column: ELISA reactivity).

Approximately 60% of the 184 peptides with the highest antibody reactivity (+++++, ++++ and +++) were further tested with another set of sera in order to determine seroconversion. This analysis was performed to support the pathogen specificity of peptide-antibody reactions, as well as to pinpoint to epitopes that may contribute to protection given low or non-detectable levels of specific antibodies in the acute phase sera, and antibody induction during convalescence. Samples from seven patients with acute-convalescent serum pairs were selected for this assay based on the seroconversion rate measured with whole MenB lysate as antigen and available sample volumes. (The convalescent sera were included in the screening pools.) The results - shown in Table 3 column Seroconversion (SC) - were calculated from the fold increase of antibody levels in convalescent samples relative to that in acute sera, as well as from the number of patients showing seroconversion (> 2 fold). Of the 109 peptides tested 55 were positive in this assay. Representative examples of peptides showing different extent of seroconversion are depicted in Fig. 3.

Example 4: Bactericidal killing activity measurements with MenB epitope-specific hyperimmune mouse sera

Experimental procedure

5

Generation of immune sera in mice.

E. coli clones harboring plasmids encoding the platform protein fused to a MenB peptide, were grown in LB medium supplemented with 50 µg/ml Kanamycin at 37°C. Overnight cultures were diluted 1:10, grown until an OD₆₀₀ of 0.5 and induced with 0.2 mM IPTG for 10 2 hours. Pelleted bacterial cells were suspended in PBS buffer and disrupted by sonication on ice, generating a crude cell extract. According to the OD₆₀₀ measurement, an aliquot corresponding to 5x10⁷ cells was injected into NMRI mice i.v., followed by a boost after 2 weeks. Serum was taken 1 week after the second injection. Epitope specific antibody levels were measured by Western blotting and peptide ELISA.

15

Results

Surface localization of bacterial antigens and their ability to induce functional (neutralizing and bactericidal) antibodies are good indicators of their protective potential. In order to 20 analyze the antibodies specific for individual epitopes and antigens without the need for recombinant protein production, we developed an effective strategy to immunize mice with the *E. coli* clones that carry immunogenic foreign epitopes selected in bacterial surface display screens. 59 *E. coli* clones were selected for the generation of mouse hyperimmune sera (see above). The presence of specific antibodies was measured by two independent 25 assays. Western blot analysis was performed by using the *E. coli* lysate as antigen and the generated antiserum as antibody source (after removal of *E. coli* specific antibodies with depletion). Immunogenicity was assessed by visual detection of immunoreactive bands corresponding to the platform proteins LamB or FhuA with altered mobility due to the presence of inserted MenB epitopes (representative examples are shown in Figs. 4 and 5). 30 We also employed peptide ELISA performed with synthetic peptides corresponding to epitopes expressed in the *E. coli* platform proteins. The levels of antibodies were assessed

by ELISA readings at two different serum dilutions (200 X and 1,000 X) measured in five individual mouse sera (representative examples are shown in Fig. 5).

Based on these two assays, two thirds of the epitopes were immunogenic in mice. Importantly, we found a good correlation between the Western blot and peptide ELISA data. The summary of these results is shown in Table 4 (clone WB and ELISA react.). Moreover, we were able to detect full length proteins by Western blot analysis with ~25% of serum samples using total bacterial lysates prepared from the MenB MC58 strain grown either in defined low-iron stress medium (RPMI) or in rich growth medium (BHI). Corresponding data are shown in Table 4, and representative examples are depicted in Fig. 6.

Serum samples that showed epitope specific antibody levels were further analysed in the *in vitro* colorimetric bactericidal assay described above. Due to the low signal background ratio obtained with whole serum, we purified total IgG in order to provide higher amounts of antibodies in this assay. 100 cfu of Meningococcus serogroup B MC58 strain were incubated with 10 or 20 µg total IgGs in the presence of guinea pig serum as complement source. In this assay the same amount of total IgGs purified from mouse sera generated with total MenB lysate showed 100% killing activity. Bactericidal activity of individual IgG preparations were calculated as percentage of killing relative to the negative control (no IgG, 0%) and positive control (hyperimmune IgG, 100%). IgGs induced by "empty" LamB and FhuA platform protein carrying *E. coli* bacteria were used as background controls that resulted in about 10% killing activity (Fig. 7). All of the sera that induced >20% bactericidal activity were considered positive, and were quantified based on the percent of reduction in bacterial growth. The results are also shown in Table 4, column BA. In summary, 10 clones or combination of clones showed pronounced bactericidal activity (> 40%; +++ or ++++), 18 and 10 were associated with intermediate (30-40%; ++) or low (20-30%; +) activity, respectively. Importantly, the bactericidal activity was IgG concentration dependent and the assay was highly reproducible. Moreover, high bactericidal activity as measured with the colorimetric assay was highly correlated with cfu when plating assay was performed.

Table 1. Serum samples for antigen identification.

Donor	Category	Pathogen*	Acute & convalescent paired samples**	Single serum samples
Meningitis patient	children	MenB; MenC	61 pairs	7
Meningitis patient	adults	MenB; MenC	14 pairs	-
Healthy exposed (household members)	carrier	MenB; MenC	N.a.	8
Healthy exposed (household members)	Non-carrier	exposed to MenB or MenC	N.a.	125
Total			150	140

Table 2: Summary of immune characterization of selected serum samples.

Serum pool #19, serogroup B infected, convalescent sera with seroconversion, high SBA and medium ELISA									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1509 2*	>64	64	0	7250	15 3	neg	neg	neg	B NT P1 1, P1 7
P1520 2*	16-64	~64	0	5000	12 7	192	192	24	B
P1526 2*	16-64	~16	0	5000	2 2	96	24	6	B 15 P1 7, P1 16
P1507 2*	16-64	~16	0	2800	23 0	12	12	24	B NT NST
Serum pool #20, serogroup B infected, convalescent sera with highest ELISA and seroconversion									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1481 2*	<16	>16	>16	9204	16 5	192	192	96	B
P1483 2*	16-64	32	>16	8754	2 5	96	96	48	B 22 NST
P1482 2*	<16	<<16	n d	10004	3 0	48	24	24	B NT NST
P1540 2	16-64	32	>16	9206	53 8	384	24	48	B 21 P1 15
P1512 2*	<16	<<16	n d	9189	7 5	96	48	48	B x C
P1539 2*	<<16	<<16	n d	7281	62 6	48	24	24	B 22 P1 10
P1532 2*	<<16	<<16	n d	7113	26 1	12	24	6	B
Serum pool #21, serogroup C infected, convalescent sera with high SBA (Prague) and high/med ELISA									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1476 2	>64	neg ^{II}	0	6000	7 7	96	neg	neg	C
P1510 2*	16-64	~16	0	4500	8 1	24	24	364	C
P1515 2	>64 ab ^{VI}	>256	>64	10128	47 7	48	96	48	C 2a P1 2, P1 5
P1474 2	<<16	n d	n d	9624	18 2	48	48	364	C 2a P1 2, P1 5
P1488 2	<16	n d	n d	6244	5 4	24	48	192	C 2a P1 2, P1 5
Serum pool #22, serogroup C infected, convalescent sera with highest ELISA with seroconversion against serogroup B									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1511 2*	16-64	n d	n d	7819	27 1	384	6	24	C 2a P1 2, P1 5
P1492 2*	<<16	n d	n d	7564	10 2	12	12	12	C
P1490 2	16-64	n d	n d	7229	8 4	96	24	364	C NT P1 2, P1 5
P1525 2	<16	n d	n d	4993	40 8	96	12	384	C 2a P1 15
P1528 2	<<16	n d	n d	4988	15 2	24	6	192	C
P1508 2*	<16	n d	n d	4184	108 0	24	neg	48	C
Serum pool #23, Exposed colonized									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1557*	<<16	<16	n d	2012	n d	6	48	12	B NT NST
P1559*	<<16	<16	n d	5552	n d	6	192	neg	C 2a P1 2, P1 5
P1560*	~64	>128	n d	448	n d	24	192	24	B 21 P1 7
P1562*	~64	<32	n d	2913	n d	24	24	12	B 4 P1 15
Serum pool #24, Exposed non-colonized									
Serum-No	SBA 1st	SBA 2nd	SBA w/o C'	ELISA U	SC	SE2A	SE2B	SE2C	phenotype
P1566*	<<16	<16	0	3174	n d	96	48	364	n d
P1582	~64	32	0	4389	n d	96	neg	neg	n d
P1604*	>64	128-256	0	1205	n d	364	192	96	n d
P1605*	>64	64	0	2150	n d	96	192	24	n d
P1607	>64	~64	0	3035	n d	12	364	192	n d

Table 3. Immunogenicity of epitopes in peptide ELISA - seroconversion.

ELISA reactivity: ELISA reactivity of peptides with human sera.

Peptide name	Amino acid (aa) seq	ELISA reactivity	Sero- conversion	SEQ ID NO:
ELISA reactivity: positive and seroconversion: positive				
NRF004.1	vkqhvsvetkttivtskptq	++++	++++	1
NRF004.4	yigarvayghvkhqvhsvetkttiv	+++	+	2
NRF004.5	kttivtskptqgaaggpi	++++	+	3
NRF004.6	aqggpiiqt dpskppyhe	+++	+++	4
NRF005.1	pwvgagggravccydtgvp	++++	++	5
NRF007.1	kriaavkpvyvfanntsisrsplree	++	++	6
NRF008.3	hqyctnqngmeqnpfratshl	+++	+	7
NRF009.1	hmaqkpnllldrlairnr	++++	+	8
NMB0035.1	vagskisgeedryshtdl	+++	+++	9
NMB0045.4	avatteehklgwaarlkqglts	++	+	10
NMB0072.1	qsevqmglvsklqdelivigtql	+++	++	11
NMB0072.3	qipglqareqslrkeidqqlrai s	+++	+	12
NMB0276.1	ppdtpfflrngnadtiaakf	+++	++	13
NMB0475.1	idrfaghlwklyqtlgnygrqvk	+++	++	14
NMB0475.2	ygrqvkermlgwgslygyhev	+++	+	15
NMB0545.2	slqaqqdetaaqvqalndevhalqt	+++	+++	16
NMB0554.2	evqrdiesmpfeii kanngdawvk	++	+	17
NMB0554.3	gdawvkaqgkelsppqisaevlr	+++	++	18
NMB0594.1	etsnmalesklklpflgnlvednqs	+++	++	19
NMB0647.4	keslyylnkhpdlvasylk	+++	+	20
NMB0700.1	ynpyaenrrrvk papspatnta	+++	++++	21
NMB0700.4	ralhnpdwyendyei pldalede	+++	++++	22
NMB0700.6	slannhvd lgalrytik tengitrlly	++++	+	23
NMB0956.2	aaaeapaaapaaapaaapaatqnn	+++	+++++	24
NMB0964.1	tpryirigvsgdyvrgrlknlp	+++	++	25
NMB0992.6	ekkraasvkdvlnagwnikgvpqgtt	+++	+	26
NMBI 108.4	lkpamdnrkql deninrvsr	++++	+++++	27
NMB 1250.4	atksspatsilpnppskstfktcs	+++	++	28
NMB 1274.1	vwfsqnpinaywq qtyhrnsplepl	++	+	29
NMB1419.1	nhglaaklnpsgmqvkrgr	-H-	++	30
NMB 1523.1	aaeapaaeapaaeaaatea	+++++	+++++	31
NMB 1523.2	aaeaaateapaaeapa	+++++	++++	32
NMB 1523.3	acggekaaeapaaeapaaeapate	+++++	+++++	33
NMBI 523.4	gekaaeapaaeapaaeapateapaae	+++++	+++++	34
NMB1523.5	eapaaeapaaeapaaeapaaea	+++++	+++++	35
NMBI 523.6	apaaeaaateapaaeaaateap	+++++	+++++	36
NMB1533.1	tpaaeapaseapaaeapaa	+++++	+++++	37
NMB1533.3	paaeatpaaeapaseapaaeapad	+++++	++++	38
NMB1533.5	apaseapaaeapadaaeapaaagnca	++++	+++++	39
NMB 1625.4	rrlkstlympalcayrfnafpklinn	++++	+	40

Peptide name	Amino acid (aa) seq	ELISA reactivity	Seroconversion	SEQ ID NO:
NMB 1768.4	asngssnqtgnsynasfhssttt	+++	+	41
NMB 1768.6	taspavsyaiqghfkdlagqnang	++	+++	42
NMB 1768.10	kademidqlnneigrealntnr	++	+	43
NMB 1772.2	kigasalnnvsdkqwinnltv	+++	++	44
NMB 1816.2	pavigpdkkrspfnqrlrnltaaa	++++	+	45
NMB 1887.2	ytgevsaeladtgtdivlighs	++++	+++++	46
NMB 1964.1	pvksagvl vgrvgai gl	+++	+	47
NMB 1985.1	tggsfgdsgspmfydaqkqkw	+++	++	48
NMB 1998.3	vetarhaylnalnrlsrqihslk	+++	++	49
NMB 1998.4	dqaatltiltgnpvlsphevewvqwn	+++	++++	50
NMB1998.8	rqqaeeqvaqaenarpdlitfg	+++	+	51
NMB 1998.10	emaeseldkarqggdaqavetarh	++	+	52
NMB2007.2	gglskieekyrksheiflkrlaalp	+++	++	53
NMB2061.1	yflqtspiqeiatlnlgsrpsrktl	+++	+	54
NMB2135.2	drlnlnitapdlrsrfgfagls	+++	++	55
ELISA reactivity: positive and seroconversion: weak				
NRFOO 1.1	glyrkkpkphrpsfpqkresknatqq	++++	+/-	56
NRFOO 1.2	knatqqefigknrnpavipaka	++++	+/-	57
NRFOO 1.3	vipakagiqqpknhnlsakte	++++	+/-	58
NRF007.2	splreeklkrfaanqylr	++++	+/-	59
NRF008.1	grrfkssqthqerghssvgrapalqa	+++	+/-	60
NMB0050.4	lppktytnasapptsiirkcpknsk	++++	+/-	61
NMB0290.2	dcrrrgfgvarfpnpvpvadcap	++++	+/-	62
NMB0308.2	qpaggllrgrngggkfggg	++++	+/-	63
NMB0545.3	vhalqtaeqsqqavhelsnkrqvl	+++	+/-	64
NMB0554.1	vtnakntiyaakrlighkfedkevqrdr	++++	+/-	65
NMB0633.2	rlparrggtqthqpvlrsqpvlqg	++++	+/-	66
NMB0647.2	eqvkhecsstgritecrqnigriief	++++	+/-	67
NMB0700.10	slitdpktisihyiqnnddddagyyy	+++	+/-	68
NMB0700.i 1	dagyyyyrprkpiqqgdlyfknyry	++++	+/-	69
NMB0815.2	edkvarrairaadllhpeklreklda	++++	+/-	70
NMB1051.1	rfldniatriveldrgilrsypgsfs	++++	+/-	71
NMB 1250.6	psgttspnlcrrnsdgilgvlipv	++++	+/-	72
NMB 1274.2	spleplaaygwrrsgaalqenayalsd	++++	+/-	73
NMB1314.1	skdlarsmslqsvrivetiagkntmg	++++	+/-	74
NMB1314.2	gkntmgieelpndkrqdvml	+++	+/-	75
NMB 1483.2	thtivrgdtvyniskryhisqddfra	++++	+/-	76
NMB 1625.2	qltekhfktanqfvsyaglnpaiiqs	++++	+/-	77
NMB 1625.3	paiiqsgtsvrggrlsrygnrrlkst	++++	+/-	78
NMB 1697.2	vsdwtasrrtcanylmskhspplska	+++	+/-	79
NMB 1730.3	adiqqpkeepkpeekpkpe	+++	+/-	80
NMB 1779.1	ingkhisiknnggnadlknlnvhaks	++++	+/-	81
NMB 1877.1	kssfπpfirggsaappplaqlfqkq	++++	+/-	82
NMB 1897.4	lpahaaaarnakptpwtpswnrags	++++	+/-	83
NMB 1936.1	vgrelvgrwdalg φ i	+++	+/-	84

Peptide name	Amino acid (aa) seq	ELISA reactivity	Seroconversion	SEQ ID NO:
NMB1985.2	eakkqaekdnaqslaliaag	++++	+/-	85
NMB 1985.6	edepnnressyhasayswlv	++++	+/-	86
NMB1985.i 1	atpfgqaasgtpnttvrkisaptank	+++	+/-	87
NMB 1985.12	aptankptcaksvcrktsaagasascfrt	++++	+/-	88
NMB 1985.13	ascfrttgpktpsttasatrhglptapf	++	+/-	89
NMB 1998.6	hnrtrdyfilkpggnpreffplnmk	+++	+/-	90
NMB1998.7	fplnmknstswqfignnrqaae	++++	+/-	91
NMB1998.i 1	vetarhaylnalnrlsrqihslk	++++	+/-	92
NMB2061.2	asrktlariqdlraipwvfwsmq	+++++	+/-	93
NMB2135.1	rlgrniiktdggfgkkgdrlnl	++++	+/-	94
ELISA reactivity: positive and seroconversion: negative				
NRF004.3	ssslglaiydfklndkfkpyigarv	++++	-	95
NRF008.2	apalqagghrfdpvcilhqyctn	+++	-	96
NMB0072.2	viqtldqvkvavtpenpqipglq	+++	-	97
NMB0290.4	arhlhngkqtrqplgkhrpsqtpa	++++	-	98
NMB0456.2	hphrrhtvrpkphirrcqngtrp	+++	-	99
NMB0493.4	vlssgrltvrnlgrlknqngtiqaa	+++	-	100
NMB0545.1	ldyaqwrqslaaadkataqhqlaqqq	+++	-	101
NMB0594.3	gkipenttilanesylyrafdnv	+++	-	102
NMBI 163.1	veagnkkmnaapgahahlglslsr	++++	-	103
NMB 1422.2	edyvhrigtgraga	++++	-	104
NMB1536.1	yaqknpkqeykreaftmfqdlwngik	++++	-	105
NMB 1730.4	ekpkpeekpkpepkpeak	++++	-	106
NMB1730.5	pkpeekpkpeekpkpepkpeakvp	++++	-	107
NMB 1998.5	wvqwggnrpqgnaavyeyinphnrtr	+++	-	108
NMB2007.1	qtlskdrhflrsafknknkygglski	++++	-	109
ELISA reactivity: positive and seroconversion: not determined				
NRF004.2	tskptqgaaggpiiqtdps	++	n.d.	110
NMB0030.1	kflnleaitwantrdtlgkhank	++	n.d.	111
NMB0030.2	hainkyehlmqrveqkqvddliea	++	n.d.	112
NMB0030.3	dlieankqsiaaaaapaaegkyekvae	+++	n.d.	113
NMB0045.1	vesavetvsgaveqvketvaemlsea	++	n.d.	114
NMB0045.2	emlseaeaaekaaeqveaakeavae	++	n.d.	115
NMB0045.3	keavaetvgeavgqvqeavatte	++	n.d.	116
NMB0050.1	ktytnasapptsirkcpknsk	++	n.d.	117
NMB0050.2	pknsktptssastacskcrdk	++	n.d.	118
NMBO050.3	kcrdkpaatppkpcaqaktftana	+++	n.d.	119
NMB0050.5	pknsktptssastacskcrdkpaa	++	n.d.	120
NMB0050.6	dkpaatppkpcaqaktftanasa	++	n.d.	121
NMBO 193.1	qvkyqgyidrqnneidsrrdie	++	n.d.	122
NMBO 193.2	srrdietklpddgidygkvkgl	++	n.d.	123
NMBO 193.3	vkgl saevqqklngkpetvggas	++	n.d.	124
NMB0214.1	knspefdtlspaqtlnhdrlrd	++	n.d.	125
NMB0249.1	rrvpleneainecwlsdrdrfayeg	++	n.d.	126
NMB0249.2	fayeglyhesrlknkpikqggewmdv	+++	n.d.	127

Peptide name	Amino acid (aa) seq	ELISA reactivity	Sero- conversion	SEQ ID NO:
NMB0249.3	ewmdvdwktaleyvrsaieciakdgk	-H-	n.d.	128
NMB0266.2	hdghgdddhghaahqhnkqdkisr	++	n.d.	129
NMB0290.1	tlrsgrtdfgqpprrfdcr	+++	n.d.	130
NMB0290.3	adcaptggnricparlarfpe	++	n.d.	131
NMB0290.5	sqtpapqqppqpdrsrgril	++	n.d.	132
NMB0456.3	ngtrpaihrrtdmavahlhppdagk	++	n.d.	133
NMB0581.1	wlaeqaenmgveiypgfaaaevlyhed	++	n.d.	134
NMB0581.2	vlyhedgsvkgiatgnmgigkdg	++	n.d.	135
NMB0581.3	gigkdgeptdsfpgmelwaqq	++	n.d.	136
NMB0647.3	griieftqdkrfsrfdlkkeslyy	++	n.d.	137
NMB0671.1	ivlpvhlprcvgcrrddhq	++	n.d.	138
NMB0671.2	vlpvhlprcvgcrrddhqrnetrl	++	n.d.	139
NMB0700.5	krrghrsvqqnnveiaaqaaelarrqqeer	++	n.d.	140
NMB0700.13	engqtenndwilmgstqeeakkn	++	n.d.	141
NMB0700.15	ndyeeipldalededvsesvdsdkq	++	n.d.	142
NMB0700.16	dsdkqpqdnlehekvetvslqpra	++	n.d.	143
NMB0749.1	rkhdtpqhphshyrrsdslitvcpr	++	n.d.	144
NMB0763.2	etaraiaekgilvgissgaavw	++	n.d.	145
NMB0772.1	riwkkktkaaadmcpaica	+++	n.d.	146
NMB0772.2	icrtaglrsngisvhnidta	++	n.d.	147
NMB0956.3	aatqnaampaaaklaaetgvd	++	n.d.	148
NMB0956.4	aetgvdvnaalqgsgrdgrvlk	++	n.d.	149
NMB 105 1.3	veaehnrldkfhaqeeawirk	++	n.d.	150
NMB 1250.1	sphlplvnwkswaispqdatkss	+++	n.d.	151
NMB 1250.2	atksspatsilpnppskstfktcs	+++	n.d.	152
NMB1250.3	fktcsanstsaagc ϕ pftpsgtsp	+++	n.d.	153
NMB1250.5	fktcsanstsaagc ϕ pftpsgtt	++	n.d.	154
NMB 1309.2	kgicsakqgqfglaeaylkrslaaq	++	n.d.	155
NMB1368.1	pkgkggwgdrgsggwrgdhkpske	+++	n.d.	156
NMB1368.2	kpskefggktrgegfkkegfkrd	+++	n.d.	157
NMB1368.3	gfkrdgfkktgegfkgrkagdsf	+++	n.d.	158
NMB1434.1	lpptappysadaisatntsksvrtpf	-H-	n.d.	159
NMB 1434.2	vrtpfspiwtsppaassakyrstta	++	n.d.	160
NMB 1434.3	ttstatgqaipptprassaaatsarvf	-H-	n.d.	161
NMB1434.4	aactnplpptappysadaisa	++	n.d.	162
NMB1434.5	daisatntsksvrtpfspiwts	-H-	n.d.	163
NMB 1434.6	piwtssppaassakyrstt	-H-	n.d.	164
NMB 1688.4	gtifas ϕ ktqpfqtasvpi	++	n.d.	165
NMB 1727.2	phatskntmskpsanssdkp	++	n.d.	166
NMBI 735.2	ptrqsaksaptsasktpc	-H-	n.d.	167
NMB 1759.3	ygislnarsdlsklgeyrflvpfks	-H-	n.d.	168
NMBI 768.2	lnnqgklsqtgsqklhidaqgkmdnr	-H-	n.d.	169
NMB 1768.3	gkmdnrgmglqdtaptasngss	++	n.d.	170
NMB 1768.5	nmlasglaaptqsgagiaaatas	-H-	n.d.	171
NMBI 768.7	gqnangkltasqetahv	-H-	n.d.	172
NMB1768.8	wqatitrefgkdiavkvgnshes	-H-	n.d.	173

Peptide name	Amino acid (aa) seq	ELISA reactivity	Sero- $\rightarrow$ conversion	SEQ ID NO:
NMB 1768.9	hesgekinysirrnlslldkademi	++	n.d.	174
NMB1887.3	vlighserslyfgekneiqrkm	++	n.d.	175
NMB 1897.2	tawvrrwqkcpsftklpahaaaaimak	++	n.d.	176
NMB 1897.8	ekrradrplrrqpaqrrqagsv	++	n.d.	177
NMB 1949.1	ttarnlaaalgsfpdgggtq	++	n.d.	178
NMB1985.5	qnnypdrviragrqywrddedepnn	++	n.d.	179
NMB1985.7	yswlvggntfaqngsgggtvnl	++	n.d.	180
NMB1985.8	lsenlnftlqnehvdagawryqlrk	++	n.d.	181
NMB1985.10	qelsdklgkaeakkqaekdnaqs	++	n.d.	182
NMB 1998.1	adtndltlfeteldtyiervemaese	++	n.d.	183
NMB 1998.2	emaeseldkarqggdaqavetarh	++	n.d.	184

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Table 4: Characteristics of MenB peptides.

WB: Western Blot with antiserum generated in mice against the indicated bacterial clones with *N. meningitidis* grown in RPMI or BHI medium; if different results for RPMI and BHI were obtained, 1 and 2 indicate results in RPMI and BHI, respectively

BA: Bactericidal activity measured with antiserum generated in mice against the indicated bacterial clones (at least 1 bacterial clone)

Clone WB: Western Blot with antiserum generated in mice against the indicated bacterial clones with *E. coli* lysate expressing the respective peptide encoded by the bacterial clone

ELISA react.: ELISA reactivity of peptides with antiserum generated in mice against the indicated bacterial clones.

SC: Seroconversion measured with synthetic peptides and human sera

Clone	Amino acid (aa) sequence	SEQ ID NO:	Clone WB	Peptide name	SEQ ID NO:	ELISA react.	WB	BA	SC
Bactericidal activity: positive									
MBFAE70	glyrkpkph ϕ sfpdkresknatqefgknrmptavipakagiqqpknhmlsakte	185	+	NRFOO 1.1	56	++	-	++	+/-
				NRFOO 1.2	57	-			+/-
				NRFOO 1.3	58	+			+/-
MBLBE10	Vkhqhsvetktivtskptqgaaggpiiqtdps	186	-	NRFO04.1	1	-	-	++	++++
				NRFO04.2	2	++			n.d.
MBFBH40	Ssslglsaiydfklndkfkpyigarvayghvkhqhsvetktivtskptqgaaggpiiqtdp skppyhe	187	++	NRFO04.3	95	+			-
				NRFO04.4	2	-			+
				NRFO04.5	3	+			+
				NRFO04.6	4	+++			+++
MBLCD93	Pwvgagggravccydgvp	5	-	NRFO05.1	5	+	-1 ++2	++	++
MBFBZ79	grrfkssqthqerghssvgrapalqagghrfdpvcvlhgyctnqngmeqnpfratshl	188	+	NRFO08.1	60	+	-	+++	+/-
				NRFO08.2	96	++++			-
				NRFO08.3	7	++++			+
MBLAA68	Hmaqkpnllrlairnr	8	+	NRFO09.1	8	-	-	++	+
MBLAK90	Vagskisgeedryshdl	9	-	NMB0035.1	9	++	-	+	+++
MBFBL65	Vesavetvsgaveqvketvaemlseaeeaaakeaaqveaakeavetvsgavgvqeava tteehklgwaarlkgltks	189	++	NMB0045.1	114	++++	+	+++	n.d.
				NMB0045.2	115	++++			n.d.

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Clone	Amino acid (aa) sequence	SEQ ID NO:	Clone WB	Peptide name	SEQ ID NO:	ELISA react.	WB	BA	SC
				NMB0045.3	116	+++			n.d.
				NMB0045.4	10	+++++			+
MBFAV94	qsevmglsklqdelivltqldqvkvavtpenpqpqlqareqslrkeidqqlrais	190	++	NMB0072.1	11	-	+	+	++
				NMB0072.2	97	-			-
				NMB0072.3	12	+++			+
MBFCD77	qvkyqgyidrqnecidsrrdieltklpdgidykvkglsvaeqqklhnhkpetvgqas	191	+	NMB0193.1	122	++++	-	++	n.d.
				NMB0193.2	123	+++			n.d.
				NMB0193.3	124	+			n.d.
MBFBG39	Knspeftlspaqtklnhldlrdflvlsgealppeqqaeklqttegaqlsakfsqnvldatdagi	192	+	NMB0214.1	125	+++	+	+	n.d.
MBFBY3 1	Itsaaatlslsapalahlhdghgddldghaaahqhnkqdkliisraqaekaalaryggkitdi	193	++	NMB0266.2	129	+++++	-	+++	n.d.
MBFBO59	vlsgrltvmlgrlknqngtiqaarlldmstggldntgnitqtgsqalldlvsagkfdn	194	++	NMB0493.4	100	-	-	++	-
MBFBS85	npvstpaknyavvgriqtgafdnagnsinaggqidiaaqnglgnsgslnaaklrsvgsd	195	+						
MBFAD49	Ldyaqwrqslaaadkataqhsqldaqddetaaqvqlndevhalqtaeqsqqqavhelsnkrgvl	196	+	NMB0545.1	101	+++++	- ¹ + ²	+	-
				NMB0545.2	16	+++++			+++
				NMB0545.3	64	++			+/-
MBFBL85	Vnakntiyaakrlighkfedkevqrdesmpfeikaanngdawvkaqkelsppqisaevl	197	+	NMB0554.1	65	-	+ ¹ - ²	+++	+/-
				NMB0554.2	17	+++++			+
				NMB0554.3	18	+++			++
MBFAP42	Wlaeqaenmgveiypgfaaaevlyhedgsvkgiatgnmgigkdgeptsfqpqmelwaqq	198	-	NMB0581.1	134	++++	+	++	n.d.
				NMB0581.2	135	-			n.d.
				NMB0581.3	136	-			n.d.
MBFBH79	etsnmalekesklplflgnlvnednqsiakngqvtlsadgkipenttilanesylrafdnv	199	+	NMB0594.1	19	+++++	+	+	++
				NMB0594.3	102	-			-
MBLAF61	Ynpyaenrrrvkppaspatnta	21	-	NMB0700.1	21	-	-	++	++++
MBLAK06	Kvnlgkngdevcvrsvdytygtnt	200	-						
MBLAW77	Tlpappspfdssayaapralhnpdwiyendyeipldalede	201	-	NMB0700.4	22	++			++++
MBLBF02	Krrghrsvqqnnveiaqaqaqlarrqeer	140	-	NMB0700.5	140	+++			n.d.
MBFAC41	Slannhvdilgalrytiktengitrllynpyaenrrrvkppaspatntasdaqkatqtdgaqiakpqnivvappspqanqaee	202	+	NMB0700.6	23	+	-	++	+
MBFAU 14	Slitdpktisihyiqnnddddagyyyyyrrprkpiqpqgdlyfknryyalksggsnampme	203	+	NMB0700.10	68	-			+/-

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Clone	Amino acid (aa) sequence	SEQ ID NO:	Clone WB	Peptide name	SEQ ID NO:	ELISA react.	WB	BA	SC
MBFAJ40	pkpeekpkpeekpkpekpvpkpkaekpvkekpsekpaeppgnasakadse	221	++	NMB1730.4	106	++++			-
MBFBH18	TvknImvavatagvadkigasalnnsdkgwinlly	222	+	NMB1730.5	107	++++			-
MBFAX28	lnmriltcaqdsrfrpnnngaytgevsaeladgtidvlgsherslyfgekneiqtrkm	223	-	NMB1772.2	44	-	-	+	++
				NMB1887.2	46	++++	-	++	++++
				NMB1887.3	175	-			n.d.
MBLBG75	Vgrelvgrvvdalg φ i	84	+	NMB1936.1	84	++++	+	++	+/-
MBLBG47	Ttdamlaaalgsfpdgggtq	178	-	NMB1949.1	178	++++	-	+++	n.d.
MBLBC64	Tggsfgdsgspmfidydaqkql	224	+	NMB1985.1	48	++++	-	+++	++
MBLAK94	Eakkaekdnaqlsal iaag	225	-	NMB1985.2	85	++			+/-
MBLBB93	Hyrsqdfrayrqtdlrqigmqknlgsgrvgilfshnrten	226	-						
MBFAX61	adndltlfereldtyiervemaeseldkarqggdaqavetarhaylnalnrlsrqihslk	227	++	NMB1998.1	183	++++	-	+	n.d.
				NMB1998.2	184	++++			n.d.
				NMB1998.3	49	-			++
MBFBP07	Dqaatlilgnpvlsphevewvqwgnrpqgnaavyeyinphnmrtdyfilkpggnpreffp lnmknsstwdfignmrqaaeqvaqaenarpdlitfig	228	-	NMB1998.4	50	+	-	+++	++++
				NMB1998.5	108	-			-
				NMB1998.6	90	-			+/-
				NMB1998.7	91	-			+/-
				NMB1998.8	51	-			+
MBFAX61	adndltlfereldtyiervemaeseldkarqggdaqavetarhaylnalnrlsrqihslk	227	++	NMB1998.10	52	++			+
				NMB1998.11	92	+			+/-
MBFAV69	qlskdrhlrsafknpnkygglskieekyrksheiflkrllaalpkefdntlpvhekleikk	229	-	NMB2007.1	109	-	+	++	-
				NMB2007.2	53	-			++
Bactericidal activity: negative									
MBFAD30	Qdgetvvadqlarvldtaataaaepaaepaaapaatqnaampaaaklaaetgvd vnaqlqgsgrdgrvlk	230	++	NMB0956.2	24	++++	+ ¹ - ²	-	++++
				NMB0956.3	148	++++			n.d.
				NMB0956.4	149	-			n.d.
MBFBIO	Rfldniatriveldrilrsypgsfsksekkaqlaveaehmrifdkfhaqeeawirk	231	++	NMB1051.1	71	-	+	-	+/-
				NMB1051.3	150	++++			n.d.
MBFBIO9	Yfdkaladpytpyianlnkgicsakqgfglaeaylkslaaqpqfpafkelartkmlag qlgdadyf	232		NMB1309.2	155	-	-	-	n.d.
MBFAC44	Skdlarsmslqsvrivetiagkntmgelpndkrqdvmlseilsspvfaeaksklt	233	+	NMB1314.1	74	-	+	-	+/-
				NMB1314.2	75	++++			+/-

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Clone	Amino acid (aa) sequence	SEQ ID NO:	Clone WB	Peptide name	SEQ ID NO:	ELISA react.	WB	BA	SC
MBFAM26	Ymlqtipsigkdtasvlyaqktekhftanqfvsyaglnpailqsgtsvrggrlsrygnrllkst lympalcayrnfafpklinn	234	-	NMB1625.2	77	-	-	-	+/-
				NMB1625.3	78	-			+/-
				NMB 1625.4	40	-			+

Table 5: Summary of the characteristics of synthetic peptides and the respective clones

Name: Name of the ORF and the respective peptide(s) (indented)

5 SEQ ID NO: SEQ ID NO: of the synthetic peptides and the clones used for determination of ELISA reactivity / seroconversion and of bactericidal activity, respectively. Data for ELISA reactivity, seroconversion and bactericidal activity are derived from tables 3 and 4

10 Group I: Bactericidal activity: positive Seroconversion: positive (incl. weak)
 Group II: Bactericidal activity: positive Seroconversion: negative
 Group III: Bactericidal activity: positive Seroconversion: not determined (n.d.)
 Group IV: Bactericidal activity: negative Seroconversion: positive (incl. weak)
 Group V: Bactericidal activity: negative Seroconversion: negative
 15 Group VI: Bactericidal activity: negative Seroconversion: not determined (n.d.)
 Group VII: Bactericidal activity: not determined (n.d.) Seroconversion: positive (incl. weak)
 Group VIII: Bactericidal activity: not determined (n.d.) Seroconversion: negative
 Group IX: Bactericidal activity: not determined (n.d.) Seroconversion: not determined (n.d.)

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NRF001	185			++	
NRF001.1	56	++++	+/-		I
NRF001.2	57	++++	+/-		I
NRF001.3	58	++++	+/-		I
NRF004	186, 187			++	
NRF004.1	1	++++	++++		I
NRF004.2	110	+++	n.d.		III
NRF004.3	95	++++	-		II
NRF004.4	2	+++	+		I
NRF004.5	3	++++	+		I
NRF004.6	4	+++	+++		I
NRF005	5			++	
NRF005.1	5	++++	++		I
NFR007				n.d.	
NRF007.1	6	++	++		VII
NRF007.2	59	++++	+/-		VII
NRF008	188			+++	
NRF008.1	60	+++	+/-		I
NRF008.2	96	+++	-		II
NRF008.3	7	+++	+		I
NRF009	8			++	
NRF009.1	8	++++	+		I
NMB0030				n.d.	
NMB0030.1	111	++	n.d.		IX
NMB0030.2	112	++	n.d.		IX
NMB0030.3	113	+++	n.d.		IX
NMB0035	9			+	

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB0035.1	9	+++	+++		I
NMB0045	189			+++	
NMB0045.1	114	++	n.d.		III
NMB0045.2	115	++	n.d.		III
NMB0045.3	116	++	n.d.		III
NMB0045.4	10	++	+		I
NMB0050				n.d.	
NMB0050.1	117	++	n.d.		IX
NMB0050.2	118	++	n.d.		IX
NMB0050.3	119	+++	n.d.		IX
NMB0050.4	61	++++	+/-		VII
NMB0050.5	120	++	n.d.		IX
NMB0050.6	121	++	n.d.		IX
NMB0072	190			+	
NMB0072.1	11	+++	++		I
NMB0072.2	97	+++	-		II
NMB0072.3	12	+++	+		I
NMB193	191			++	
NMB0193.1	122	++	n.d.		III
NMB0193.2	123	++	n.d.		III
NMB0193.3	124	++	n.d.		III
NMB0214	192			+	
NMB0214.1	125	++	n.d.		III
NMB0249				n.d.	IX
NMB0249.1	126	++	n.d.		IX
NMB0249.2	127	+++	n.d.		IX
NMB0249.3	128	++	n.d.		IX
NMB0266	193			+++	
NMB0266.2	129	++	n.d.		III
NMB0276				n.d.	
NMB0276.1	13	+++	++		VII
NMB0290				n.d.	
NMB0290.1	130	+++	n.d.		IX
NMB0290.2	62	++++	+/-		VII
NMB0290.3	131	++	n.d.		IX
NMB0290.4	98	++++	-		VIII
NMB0290.5	132	++	n.d.		IX
NMB0308				n.d.	
NMB0308.2	63	++++	+/-		VII
NMB0456				n.d.	
NMB0456.2	99	+++	-		VIII
NMB0456.3	133	++	n.d.		IX
NMB0475				n.d.	
NMB0475.1	14	+++	++		VII

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB0475.2	15	+++	+		VII
NMB0493	194, 195			++	
NMB0493.4	100	+++	-		II
NMB0545	196			+	
NMB0545.1	101	+++	-		II
NMB0545.2	16	+++	+++		I
NMB0545.3	64	+++	+/-		I
NMB0554	197			+++	
NMB0554.1	65	++++	+/-		I
NMB0554.2	17	++	+		I
NMB0554.3	18	+++	++		I
NMB0581	198			++	
NMB0581.1	134	++	n.d.		III
NMB0581.2	135	++	n.d.		III
NMB0581.3	136	++	n.d.		III
NMB0594	199			+	
NMB0594.1	19	+++	++		I
NMB0594.3	102	+++	-		II
NMB0633				n.d.	
NMB0633.2	66	++++	+/-		VII
NMB0647				n.d.	
NMB0647.2	67	++++	+/-		VII
NMB0647.3	137	++	n.d.		IX
NMB0647.4	20	+++	+		VII
NMB0671				n.d.	
NMB0671.1	138	++	n.d.		IX
NMB0671.2	139	++	n.d.		IX
NMB0700	21, 140, 200-204			++	
NMB0700.1	21	+++	++++		I
NMB0700.4	22	+++	++++		I
NMB0700.5	140	++	n.d.		III
NMB0700.6	23	++++	+		I
NMB0700.10	68	+++	+/-		I
NMB0700.11	69	++++	+/-		I
NMB0700.13	141	++	n.d.		III
NMB0700.15	142	++	n.d.		III
NMB0700.16	143	++	n.d.		III
NMB0749				n.d.	
NMB0749.1	144	++	n.d.		IX
NMB0763				n.d.	
NMB0763.2	145	++	n.d.		IX
NMB0772				n.d.	
NMB0772.1	146	+++	n.d.		IX

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB0772.2	147	++	n.d.		IX
NMB0815	205, 206			++	
NMB0815.2	70	++++	+/-		I
NMB0956	230			-	
NMB0956.2	24	+++	+++++		IV
NMB0956.3	148	++	n.d.		VI
NMB0956.4	149	++	n.d.		VI
NMB0964	207			++	
NMB0964.1	25	+++	++		I
NMB0992	208, 209			+++	
NMB0992.6	26	+++	+		I
NMB1051	231			-	
NMB1051.1	71	++++	+/-		IV
NMB1051.3	150	++	n.d.		VI
NMB1108	210			++	
NMB1108.4	27	++++	+++++		I
NMB1163	211, 212			++	
NMB1163.1	103	++++	-		II
NMB1250				n.d.	
NMB1250.1	151	+++	n.d.		IX
NMB1250.2	152	+++	n.d.		IX
NMB1250.3	153	+++	n.d.		IX
NMB1250.4	28	+++	++		VII
NMB1250.5	154	++	n.d.		IX
NMB1250.6	72	++++	+/-		VII
NMB1274	213			+	
NMB1274.1	29	++	+		I
NMB1274.2	73	++++	+/-		I
NMB1309				n.d.	
NMB1309.2	155	++	n.d.		IX
NMB1314				-	
NMB1314.1	74	++++	+/-		IV
NMB1314.2	75	+++	+/-		IV
NMB1368				n.d.	
NMB1368.1	156	+++	n.d.		IX
NMB1368.2	157	+++	n.d.		IX
NMB1368.3	158	+++	n.d.		IX
NMB1419				++	
NMB1419.1	30	+++	++		I
NMB1422	104, 214			+	
NMB1422.2	104	++++	-		II
NMB1434				n.d.	
NMB1434.1	159	++	n.d.		IX
NMB1434.2	160	++	n.d.		IX

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB1434.3	161	++	n.d.		IX
NMB1434.4	162	++	n.d.		IX
NMB1434.5	163	++	n.d.		IX
NMB1434.6	164	++	n.d.		IX
NMB1483	215, 216			+	
NMB1483.2	76	++++	+/-		I
NMB1523				n.d.	
NMB1523.1	31	+++++	+++++		VII
NMB1523.2	32	+++++	++++		VII
NMB1523.3	33	+++++	+++++		VII
NMB1523	217			++++	
NMB1523.4	34	+++++	+++++		I
NMB1523.5	35	+++++	+++++		I
NMB1523.6	36	+++++	+++++		I
NMB1533				n.d.	
NMB1533.1	37	+++++	+++++		VII
NMB1533	218, 219			++++	
NMB1533.3	38	+++++	++++		I
NMB1533.5	39	++++	+++++		I
NMB1536				n.d.	
NMB1536.1	105	++++	-		VIII
NMB1625				-	
NMB1625.2	77	++++	+/-		IV
NMB1625.3	78	++++	+/-		IV
NMB1625.4	40	++++	+		IV
NMB1688.4				n.d.	
NMB1688.4	165	++	n.d.		IX
NMB1697.2				n.d.	
NMB1697.2	79	+++	+/-		VII
NMB1727.2				n.d.	
NMB1717.2	166	++	n.d.		IX
NMB1730	220, 221			++	
NMB1730.3	80	+++	+/-		I
NMB1730.4	106	++++	-		II
NMB1730.5	107	++++	-		II
NMB1735				n.d.	
NMB1735.2	167	++	n.d.		IX
NMB1759				n.d.	
NMB1759.3	168	++	n.d.		IX
NMB1768				n.d.	
NMB1768.2	169	++	n.d.		IX
NMB1768.3	170	++	n.d.		IX
NMB1768.4	41	+++	+		VII
NMB1768.5	171	++	n.d.		IX

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB1768.6	42	++	+++		VII
NMB1768.7	172	++	n.d.		IX
NMB1768.8	173	++	n.d.		IX
NMB1768.9	174	++	n.d.		IX
NMB1768.10	43	++	+		VI
NMB1772	222			+	
NMB1772.2	44	+++	++		I
NMB1779				n.d.	
NMB1779.1	81	++++	+/-		VII
NMB1816				n.d.	
NMB1816.2	45	++++	+		VII
NMB1877				n.d.	
NMB1877.1	82	++++	+/-		VII
NMB1887	223			++	
NMB1887.2	46	++++	+++++		I
NMB1887.3	175	++	n.d.		III
NMB1897				n.d.	
NMB1897.2	176	++	n.d.		IX
NMB1897.4	83	++++	+/-		VII
NMB1897.8	177	++	n.d.		IX
NMB1936	84			++	
NMB1936.1	84	+++	+/-		VII
NMB1949	178			+++	
NMB1949.1	178	++	n.d.		III
NMB1964				n.d.	
NMB1964.1	47	+++	+		VII
NMB1985	224-226			+++	
NMB1985.1	48	+++	++		I
NMB1985.2	85	++++	+/-		I
NMB1985				n.d.	
NMB1985.5	179	++	n.d.		IX
NMB1985.6	86	++++	+/-		VII
NMB1985.7	180	++	n.d.		IX
NMB1985.8	181	++	n.d.		IX
NMB1985.10	182	++	n.d.		IX
NMB1985.11	87	+++	+/-		VII
NMB1985.12	88	++++	+/-		VII
NMB1985.13	89	++	+/-		VII
NMB1998	227			+	
NMB1998.1	183	++	n.d.		III
NMB1998.2	184	++	n.d.		III
NMB1998.3	49	+++	++		I
NMB1998	227, 228			+++	
NMB1998.4	50	+++	++++		I

Name	SEQ ID NO:	ELISA reactivity (synthetic peptides)	Seroconversion (synthetic peptides)	Bactericidal activity (clones)	Group
NMB1998.5	108	+++	-		II
NMB1998.6	90	+++	+/-		I
NMB1998.7	91	++++	+/-		I
NMB1998.8	51	+++	+		I
NMB1998.10	52	++	+		I
NMB1998.11	92	++++	+/-		I
NMB2007	229			++	
NMB2007.1	109	++++	-		II
NMB2007.2	53	+++	++		I
NMB2061				n.d.	
NMB2061.1	54	+++	+		VII
NMB2061.2	93	+++++	+/-		VII
NMB2135				n.d.	
NMB2135.1	94	++++	+/-		VII
NMB2135.2	55	+++	++		VII

Claims

- 5 1. A peptide consisting of one antigen of *N. meningitidis* of the SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 10 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 15 177, 178, 179, 180, 181, 182, 183 and/or 184 or a functional active fragment thereof.
2. The peptide of claim 1 further consisting of
- (i) 1 to 50 additional amino acid residue(s), preferably 1 to 40, more preferably 1 to 20 30, even more preferably at most 1 to 25, still more preferably at most 1 to 10, most preferably 1, 2, 3, 4 or 5 additional amino acids residue(s); and/or
- (ii) at least one amino acid residue heterologous to the antigen.
3. The peptide of claim 2, characterized in that it comprises at least 2, preferably at 25 least 3 antigens as defined in claim 1.
4. The peptide of claim 2 or claim 3, wherein the amino acid residue(s) is/are flanking the antigen C-terminally, N-terminally or C- and N-terminally.
- 30 5. The peptide of any of claims 2 to 4 comprising at least one amino acid sequence of the SEQ ID NO: 185 to 194, 196 to 199, 202 to 205, 207, 209 to 211, 213, 215, 217 to 225, 227 to 234.

6. The peptide of any of claims 1 to 5, wherein the functional active fragment consists of at least 60 %, preferably at least 70 %, more preferably at least 80 %, still more preferably at least 90 %, even more preferably at least 95 %, most preferably 99 % of the antigen of any of the SEQ ID NOS: 1 to 184.

5

7. The peptide of any of claims 1 to 6, wherein the antigen is a functional active variant of an antigen of any of the SEQ ID NOS: 1 to 184 and wherein the variant has at least 50 % sequence identity to the antigen of any of the SEQ ID NOS: 1 to 184, especially at least 60 %, preferably at least 70 %, more preferably at least 80 %
- 10 %, still more preferably at least 90 %, even more preferably at least 95 %, most preferably 99 % sequence identity to the antigen of any of the SEQ ID NOS: 1 to 184.

10

8. The peptide of claim 7, wherein the variant is derived from the antigen of any of the SEQ ID NOS: 1 to 184 by at least one conservative amino acid substitution.

15

9. A nucleic acid coding for the peptide according to any of claims 1 to 8.

10. The nucleic of claim 9, wherein the nucleic acid is located in a vector.

20

11. A pharmaceutical composition, especially a vaccine, comprising
- at least one peptide according to any of claims 1 to 8; and/or
 - at least one polypeptide comprising a peptide according to any of claims 1 to 8.

25

12. A pharmaceutical composition containing a nucleic acid selected from the group consisting of:
- (a) a nucleic acid according to claim 9;
 - (b) a nucleic acid coding for the polypeptide as defined in claim 11; or
 - (c) a nucleic acid complementary to the nucleic acid of (a) or (b).

30

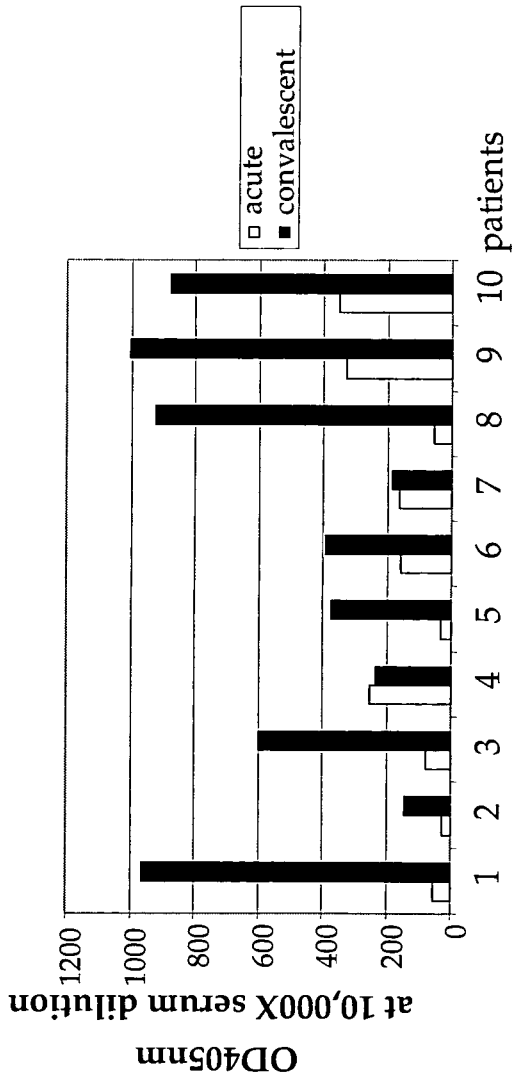
13. The pharmaceutical composition of claim 12, wherein the nucleic acid is comprised in a vector and/or a cell.

14. An antibody or functional active fragment thereof which binds specifically to the antigen of claim 1.
15. The antibody or functional active fragment thereof of claim 14, wherein the
5 antibody is a monoclonal, polyclonal, chimeric or humanized antibody, or wherein the functional active fragment comprises a Fab fragment.
16. A hybridoma cell line which produces the antibody according to claim 14 or 15.
- 10 17. A method for producing an antibody according to claim 14 or 15, characterized by the following steps:
(a) administering an effective amount of the peptide according to any of claims 1 to 8 to an animal; and
(b) isolating the antibody produced by the animal in response to the administration
15 of step (a) from the animal.
18. A method for producing an antibody according to claim 14 or 15, characterized by the following steps:
(a) contacting a B cell with an effective amount of the peptide according to any of
20 claims 1 to 8;
(b) fusing the B cell of step (a) with a myeloma cell to obtain a hybridoma cell; and
(c) isolating the antibody produced by the cultivated hybridoma cell.
- 25 19. The method of claim 17 or 18, wherein the isolated antibody is additionally purified.
20. A pharmaceutical composition, especially a vaccine, comprising the antibody according to claim 14 or 15.
- 30 21. Use of the peptide according to any of claims 1 to 8, the polypeptide as defined in claim 11, the nucleic acid according to claim 8 or 9, or the nucleic acid as defined in claim 12 or 13 for the manufacture of a medicament for the immunization of a

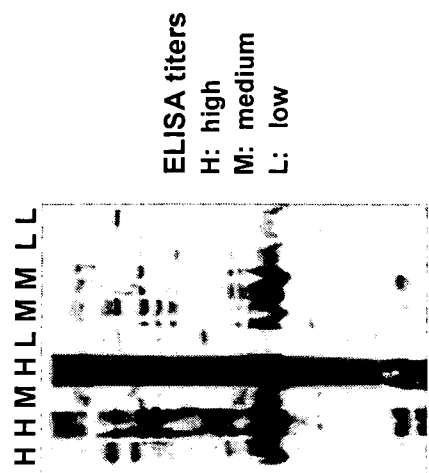
subject, preferably against *N. meningitidis*, more preferably against *N. meningitidis* serogroup B.

22. Use of the antibody or functional fragment thereof according to claim 14 or 15 for
5 the manufacture of a medicament for the treatment of an infection, preferably a *N. meningitidis* infection, more preferably a *N. meningitidis* serogroup B infection.
23. Method of diagnosing a *N. meningitidis* infection comprising the steps of:
10 (a) contacting a sample obtained from a subject with the peptide according to any of claims 1 to 8; and
(b) detecting the presence of an antibody against *N. meningitidis* in the sample.
24. Method of diagnosing a *N. meningitidis* infection comprising the steps of:
15 (a) contacting a sample obtained from a subject with the antibody according to claim 14 or 15; and
(b) detecting the presence of an antigen of *N. meningitidis* in the sample.
25. A method for identifying a ligand capable of binding to a peptide according to any
20 of claims 1 to 8 comprising:
(a) providing a test system comprising the peptide,
(b) contacting the test system with a test compound, and
(c) detecting a signal generated in response to the binding of the test compound to the peptide.
- 25 26. Use of any of the peptide according to any of claims 1 to 8 for the isolation and/or purification and/or identification of an interaction partner of the peptide.

A



B



1/1

C



Fig. 1

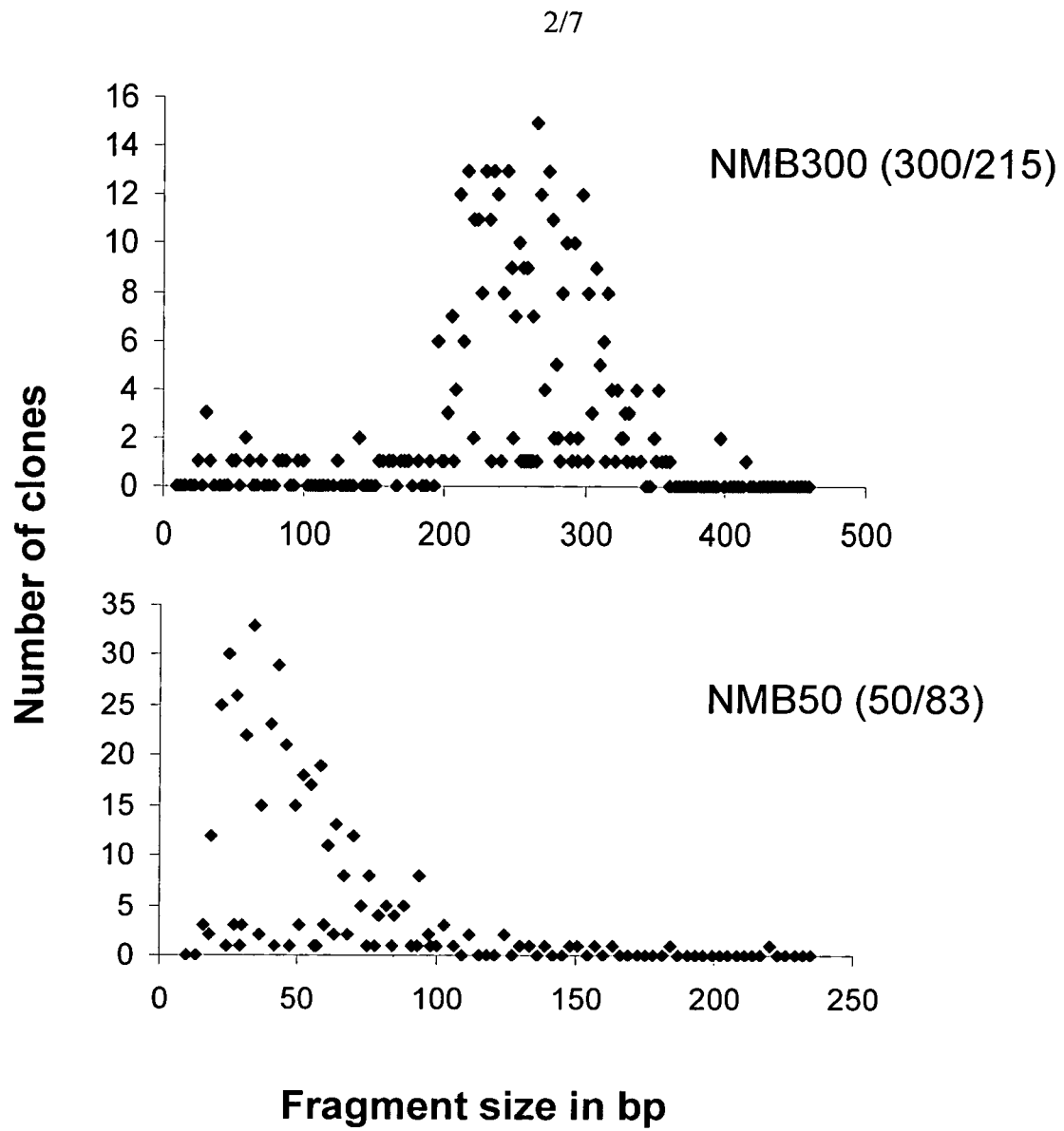


Fig. 2

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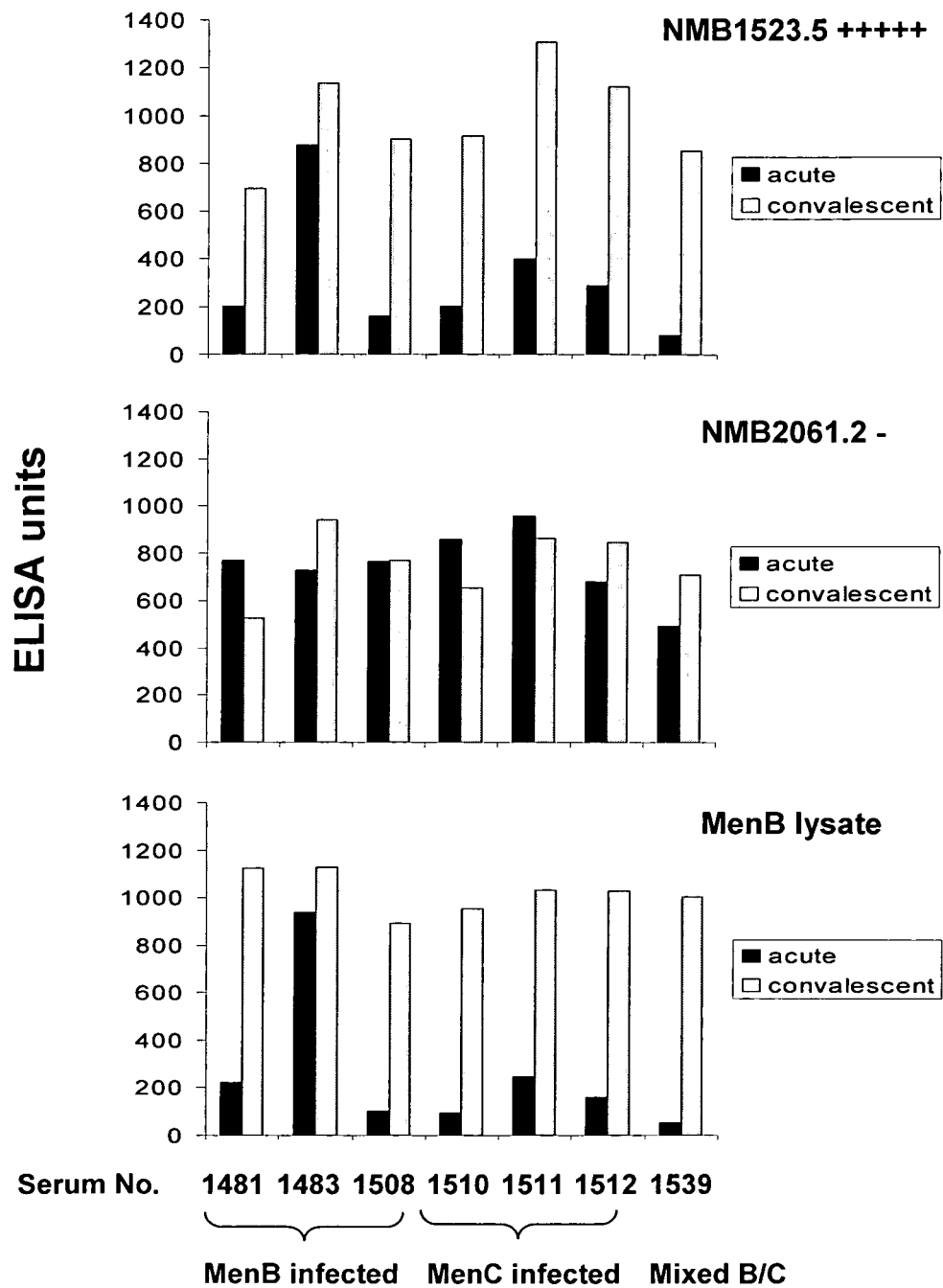


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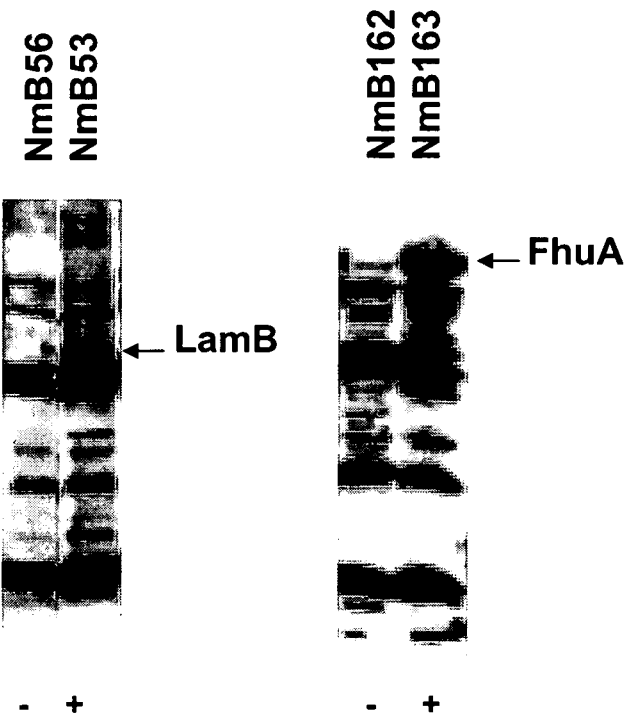


Fig. 4

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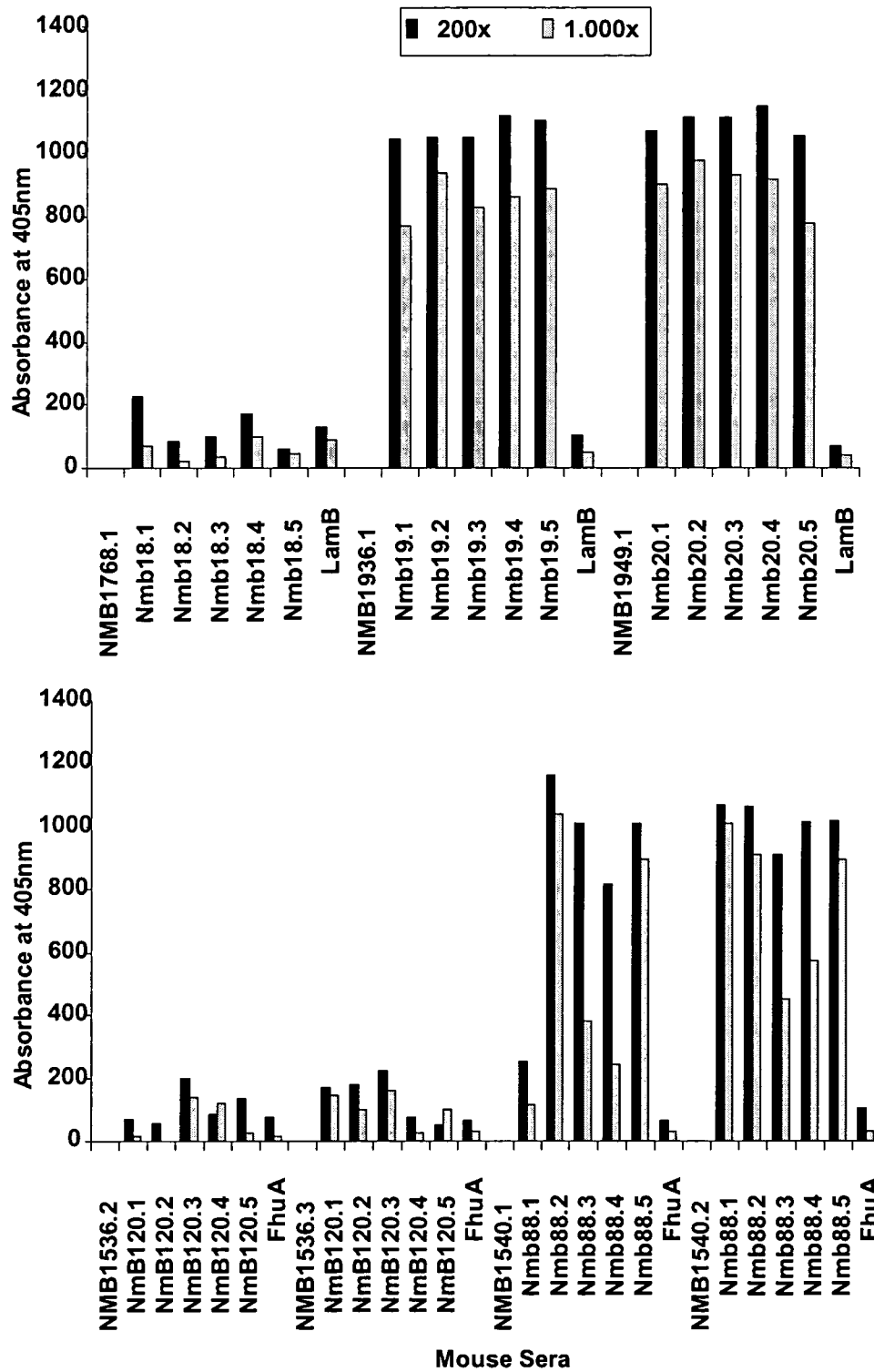


Fig. 5

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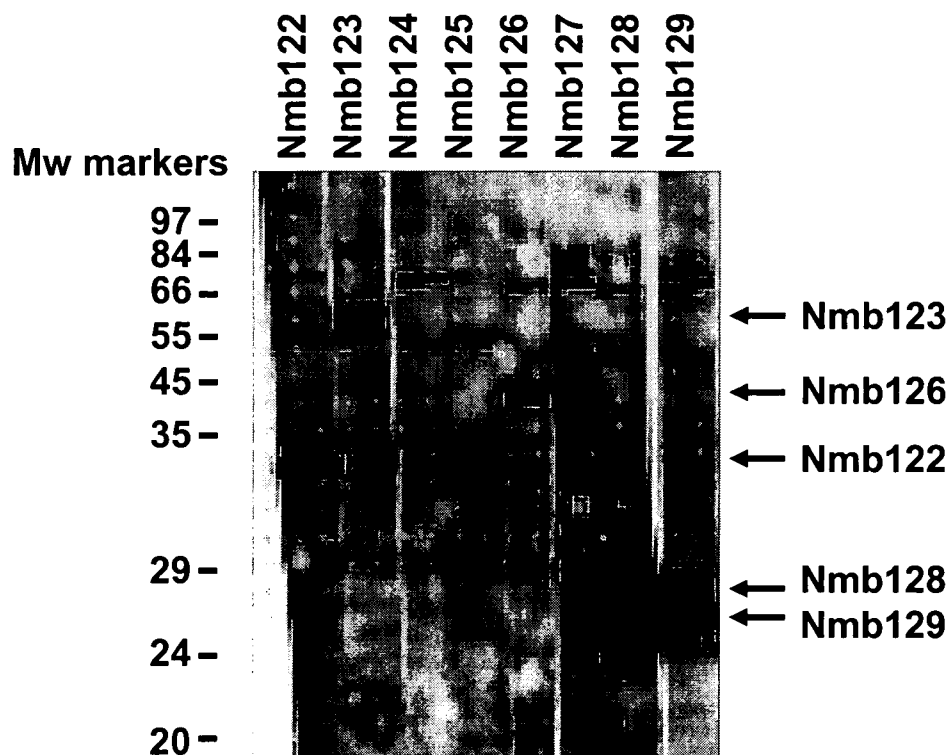


Fig. 6

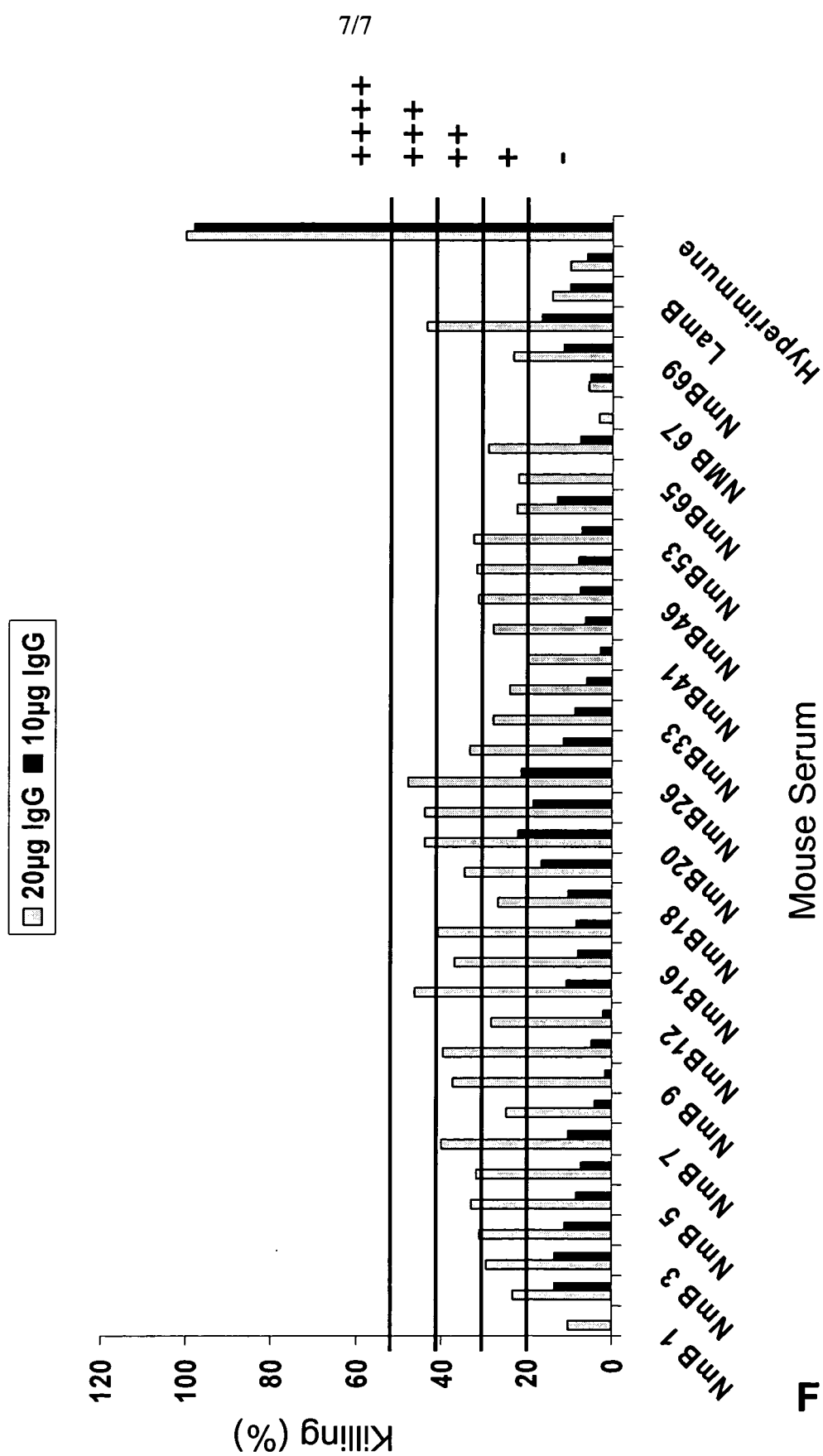


Fig. 7

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<151> 2005-10-14

<160> 234

<170> PatentIn version 3.1

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GIy Pro H e

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Ser	lie	Ser	Arg	Ser	Pro	Leu	Arg	GIu	GIu
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Asn Arg

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Asp Leu

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He Val He Gln Thr Gln Leu
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Asn Asp GIu VaI His Ala Leu GIn Thr
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Asn Asn Gly Asp Ala Trp VaI Lys
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Phe	Leu	GIy	Asn	Leu	VaI	GIu	Asp	Asn	GIn	Ser
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Tyr	Leu	Lys
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Arg Ala Leu His Asn Pro Asp Trp Tyr GIu Asn Asp Tyr GIu GIu lie
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Pro Leu Asp Ala Leu GIu Asp GIu
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Ala Ala Pro Ala Ala Thr GIn Asn Asn
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Ala Pro Ala Ala GIu Ala
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Leu	Ala	GIy	GIn	Asn	Ala	Asn	GIy
			20				

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lie	Ala	Leu	Asn	Thr	Asn	Arg
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Ala	GIn	Lys	GIn	Lys	Trp	Leu
			20			

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Arg	GIn	lie	His	Ser	Leu	Lys
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Phe Leu Lys Arg Leu Ala Ala Leu Pro
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Lys Arg GIu Ser Lys Asn Ala Thr GIn GIn
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Ala Val H e Pro Ala Lys Ala

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Leu Ser Ala Lys Thr GIu
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20 25

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<220>

<223> Peptide fragment

<400> 61

Leu Pro Pro Lys Thr Tyr Thr Asn Ala Ser Ala Pro Pro Thr Ser lie
1 5 10 15

lie Arg Lys Cys Pro Lys Asn Ser Lys
20 25

<210> 62

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 62

Asp Cys Arg Arg Arg GIy Phe GIy VaI Ala Arg Phe Pro Asn Pro VaI
1 5 10 15

Pro VaI Ala Asp Cys Ala Pro
20

<210> 63

<211> 20

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 63

GIn Pro Ala GIy GIy Leu Leu Arg GIy Arg Arg GIy Asn GIy GIy Lys
1 5 10 15

Phe GIy GIy GIy
20

<210> 64

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 64

VaI His Ala Leu GIn Thr Ala GIu GIn Ser GIn GIn GIn Ala VaI His
1 5 10 15

GIu Leu Ser Asn Lys Arg GIy VaI Leu
20 25

<210> 65

<211> 28

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 65

VaI Thr Asn Ala Lys Asn Thr lie Tyr Ala Ala Lys Arg Leu lie GIy
1 5 10 15

His Lys Phe GIu Asp Lys GIu VaI GIn Arg Asp lie
 20 25

<210> 66

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 66

Arg Leu Pro Ala Arg Arg GIy GIy Thr GIn Thr His GIn Pro VaI Leu
 1 5 10 15

Arg Arg Ser GIn Pro VaI Leu GIy GIn
 20 25

<210> 67

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 67

GIu GIn VaI Lys His GIu Cys Ser Ser Thr GIy Arg H e Thr GIu Cys
 1 5 10 15

Arg GIn Asn H e GIy Arg H e H e GIu Phe
 20 25

<210> 68

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 68

Ser Leu lie Thr Asp Pro Lys Thr lie Ser lie His Tyr lie Gln Asn
1 5 10 15

Asn Asp Asp Asp Asp Ala GIy Tyr Tyr Tyr
20 25

<210> 69

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 69

Asp Ala GIy Tyr Tyr Tyr Tyr Arg Pro Arg Lys Pro lie Pro Gln GIy
1 5 10 15

Lys Asp Leu Tyr Phe Lys Asn Tyr Arg Tyr
20 25

<210> 70

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 70

GIu Asp Lys Val Ala Arg Arg Ala lie Arg Ala Ala Asp Leu Leu His
1 5 10 15

Pro GIu Lys Leu Arg GIu Lys Leu Asp Ala
20 25

<210> 71

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 71

Arg Phe Leu Asp Asn lie Ala Thr Arg lie VaI GIu Leu Asp Arg GIy
1 5 10 15

lie Leu Arg Ser Tyr Pro GIy Ser Phe Ser
20 25

<210> 72

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 72

Pro Ser GIy Thr Thr Ser Pro Asn Leu Cys Arg Asn Arg Arg Ser Asp
1 5 10 15

GIy lie Leu GIy VaI Leu H e Pro VaI
20 25

<210> 73

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 73

Ser Pro Leu GIu Pro Leu Ala Ala Tyr GIy Trp Trp Arg Ser GIy Ala
1 5 10 15

Ala Leu GIn GIu Asn Ala Tyr Ala Leu Ser Asp
20 25

<210> 74

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 74

Ser Lys Asp Leu Ala Arg Ser Met Ser Leu GIn Ser VaI Arg lie VaI
1 5 10 15

GIu Thr lie Ala GIy Lys Asn Thr Met GIy
20 25

<210> 75

<211> 19

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 75

GIy Lys Asn Thr Met GIy lie GIu Leu Pro Asn Asp Lys Arg GIn Asp
1 5 10 15

VaI Met Leu

<210> 76

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 76

Thr	His	Thr	H e	Val	Arg	Gly	Asp	Thr	Val	Tyr	Asn	H e	Ser	Lys	Arg
1				5					10					15	

Tyr	His	H e	Ser	Gln	Asp	Asp	Phe	Arg	Ala
			20					25	

<210> 77

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 77

Gln	Leu	Thr	Glu	Lys	His	Phe	Lys	Thr	Ala	Asn	Gln	Phe	Val	Ser	Tyr
1				5					10					15	

Ala	Gly	Leu	Asn	Pro	Ala	H e	H e	Gln	Ser
			20					25	

<210> 78

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 78

Pro	Ala	H e	H e	Gln	Ser	Gly	Thr	Ser	Val	Arg	Gly	Arg	Gly	Arg	Leu
1				5					10					15	

Ser Arg Tyr Gly Asn Arg Arg Leu Lys Ser Thr

20

25

<210> 79

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 79

VaI Ser Asp Trp Thr Ala Ser Arg Arg Thr Cys Ala Asn H e Leu Pro
1 5 10 15

Met Ser Lys His Ser Pro Pro Leu Ser Lys Ala
20 25

<210> 80

<211> 19

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 80

Ala Asp lie GIn GIn Pro Lys GIu GIu Pro Lys Pro GIu GIu Lys Pro
1 5 10 15

Lys Pro GIu

<210> 81

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 81

lie	Asn	GIy	Lys	His	lie	Ser	lie	Lys	Asn	Asn	GIy	GIy	Asn	Ala	Asp
1				5					10					15	

Leu	Lys	Asn	Leu	Asn	VaI	His	Ala	Lys	Ser
			20					25	

<210> 82

<211> 28

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 82

Lys	Ser	Ser	Phe	Arg	Arg	Pro	Phe	Phe	lie	Arg	GIy	GIy	Ser	Ala	Ala
1				5					10					15	

Pro	Pro	Pro	Leu	Ala	GIn	Lys	Leu	Phe	GIn	Lys	GIn
			20					25			

<210> 83

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 83

Leu	Pro	Ala	His	Ala	Ala	Ala	Ala	Arg	Arg	Asn	Ala	Lys	Pro	Thr	Pro
1				5					10					15	

Trp	Thr	Pro	Ser	Trp	Asn	Arg	Ala	GIy	lie	Ser
			20					25		

<210> 84

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 84

VaI	GIy	Arg	GIu	Leu	VaI	GIy	Arg	VaI	VaI	Asp	Ala	Leu	GIy	Arg	Pro
1			5					10					15		

H e

<210> 85

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 85

GIu	Ala	Lys	Lys	GIn	Ala	GIu	Lys	Asp	Asn	Ala	GIn	Ser	Leu	Asp	Ala
1			5					10					15		

Leu	lie	Ala	Ala	GIy
			20	

<210> 86

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 86

GIu	Asp	GIu	Pro	Asn	Asn	Arg	GIu	Ser	Ser	Tyr	His	H e	Ala	Ser	Ala
1				5				10					15		

Tyr Ser Trp Leu Val Gly
20

<210> 87

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 87

Ala Thr Pro Phe Gly Gln Ala Ala Ser Gly Thr Pro Asn Thr Thr Val
1 5 10 15

Arg Lys Ile Ser Ala Pro Thr Ala Asn Lys
20 25

<210> 88

<211> 29

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 88

Ala Pro Thr Ala Asn Lys Pro Thr Cys Ala Lys Ser Val Cys Arg Lys
1 5 10 15

Thr Ser Ala Ala Gly Ala Ser Ala Ser Cys Phe Arg Thr
20 25

<210> 89

<211> 28

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 89

Ala	Ser	Cys	Phe	Arg	Thr	Thr	GIy	Pro	Lys	Thr	Pro	Ser	Thr	Thr	Ala
1				5					10					15	

Ser	Ala	Thr	Arg	His	GIy	Leu	Pro	Thr	Ala	Pro	Phe
			20				25				

<210> 90

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 90

His	Arg	Asn	Arg	Arg	Thr	Asp	Tyr	Phe	lie	Leu	Lys	Pro	GIy	GIy	Asn
1				5					10					15	

Pro	Arg	GIu	Phe	Phe	Pro	Leu	Asn	Met	Lys
			20				25		

<210> 91

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 91

Phe	Pro	Leu	Asn	Met	Lys	Asn	Ser	Thr	Ser	Trp	GIn	Phe	lie	GIy	Asn
1				5					10					15	

Asn	Arg	GIn	GIn	Ala	Ala	GIu
			20			

<210> 92

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 92

VaI	GIu	Thr	Ala	Arg	His	Ala	Tyr	Leu	Asn	Ala	Leu	Asn	Arg	Leu	Ser
1				5					10					15	

Arg	GIn	H e	His	Ser	Leu	Lys
			20			

<210> 93

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 93

Ala	Ser	Arg	Lys	Thr	Leu	Ala	Arg	lie	GIn	Asp	Leu	Arg	Ala	lie	Pro
1				5					10					15	

Trp	VaI	Phe	Ser	Trp	Met	GIn
			20			

<210> 94

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 94

Arg Leu GIy Arg Asn lie lie Lys Thr Asp GIy GIy Phe GIy Lys Lys
1 5 10 15

GIy Asp Arg Leu Asn Leu Asn
20

<210> 95

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 95

Ser Ser Ser Leu GIy Leu Ser Ala lie Tyr Asp Phe Lys Leu Asn Asp
1 5 10 15

Lys Phe Lys Pro Tyr lie GIy Ala Arg VaI
20 25

<210> 96

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 96

Ala Pro Ala Leu GIn Ala GIy GIy His Arg Phe Asp Pro VaI Cys Leu
1 5 10 15

His GIn Tyr Cys Thr Asn
20

<210> 97

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 97

VaI	lie	GIn	Thr	GIn	Leu	Asp	GIn	VaI	Lys	Ala	VaI	Thr	Pro	GIu	Asn
1				5					10					15	

Pro	GIn	lie	Pro	GIy	Leu	GIn
			20			

<210> 98

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 98

Ala	Arg	His	Leu	His	Arg	Asn	GIy	Lys	GIn	Thr	Arg	GIn	Pro	Leu	GIy
1				5					10					15	

Lys	His	Arg	Pro	Ser	GIn	Thr	Pro	Ala
			20					25

<210> 99

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 99

His	Pro	Pro	His	Arg	Arg	His	Thr	VaI	Arg	Pro	Lys	Pro	His	Arg	lie
1				5					10					15	

Arg Arg Cys GIn Asn GIy Thr Arg Pro

20

25

<210> 100

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 100

VaI Leu Ser Ser GIy Arg Leu Thr VaI Arg Asn Leu GIy Arg Leu Lys
1 5 10 15

Asn GIn Asn Asn GIy Thr lie GIn Ala Ala
20 25

<210> 101

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 101

Leu Asp Tyr Ala GIn Trp Arg GIn Ser Leu Ala Ala Ala Asp Lys Ala
1 5 10 15

Thr Ala GIn His GIn Ser Leu GIn Ala GIn GIn
20 25

<210> 102

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 102

GIy	Lys	lie	Pro	GIu	Asn	Thr	Thr	lie	Leu	Ala	Asn	GIu	Ser	Tyr	Leu
1				5					10					15	

Tyr	Arg	Ala	Phe	Asp	Asn	VaI
			20			

<210> 103

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 103

VaI	GIu	Ala	GIy	Asn	Lys	Lys	Met	Asn	Ala	Ala	Pro	GIy	Ala	His	Ala
1				5					10					15	

His	Leu	GIy	Leu	Leu	Leu	Ser	Arg
			20				

<210> 104

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 104

GIu	Asp	Tyr	VaI	His	Arg	lie	GIy	Arg	Thr	GIy	Arg	Ala	GIy	Ala
1				5					10				15	

<210> 105

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 105

Tyr	Ala	GIn	Lys	Asn	Pro	Lys	GIn	GIu	Tyr	Lys	Arg	GIu	Ala	Phe	Thr
1				5				10						15	

Met	Phe	GIn	Asp	Leu	Trp	Asn	GIy	lie	Lys
			20				25		

<210> 106

<211> 18

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 106

GIu	Lys	Pro	Lys	Pro	GIu	GIu	Lys	Pro	Lys	Pro	GIu	Pro	Lys	Pro	GIu
1				5				10						15	

Ala Lys

<210> 107

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 107

Pro	Lys	Pro	GIu	GIu	Lys	Pro	Lys	Pro	GIu	GIu	Lys	Pro	Lys	Pro	GIu
1				5				10						15	

Pro	Lys	Pro	GIu	Ala	Lys	Pro	VaI	Pro
			20				25	

<210> 108

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 108

Trp	VaI	GIn	Trp	GIy	Asn	Arg	Pro	GIn	GIy	Asn	Ala	Ala	VaI	Tyr	GIu
1				5					10					15	

Tyr	lie	Asn	Pro	His	Arg	Asn	Arg	Arg	Thr
		20						25	

<210> 109

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 109

GIn	Thr	Leu	Ser	Lys	Asp	Arg	His	Phe	Leu	Arg	Ser	Ala	Phe	Lys	Asn
1				5					10					15	

Pro	Asn	Lys	Tyr	GIy	GIy	Leu	Ser	Lys	lie
			20					25	

<210> 110

<211> 20

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 110

Thr Ser Lys Pro Thr Gln Gly Ala Ala Gln Gly Gly Pro lie H e Gln
1 5 10 15

Thr Asp Pro Ser
20

<210> 111

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 111

Lys Phe Leu Asn Leu GIu Ala lie Thr Trp Ala Asn Thr Arg Asp Thr
1 5 10 15

Leu GIy Lys His Ala H e Asn Lys
20

<210> 112

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 112

His Ala H e Asn Lys Tyr Glu His Leu Met Gln Arg Val Glu Gln Lys
1 5 10 15

Gln Val Asp Asp Leu H e Glu Ala
20

<210> 113

<211> 28

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 113

Asp Leu lie GIu Ala Asn Lys GIn Ser lie Ala Ala Ala Ala Ala Pro
1 5 10 15

Ala Ala GIu GIu GIy Lys Tyr GIu Lys VaI Ala GIu
20 25

<210> 114

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 114

VaI GIu Ser Ala VaI GIu Thr VaI Ser GIy Ala VaI GIu GIn VaI Lys
1 5 10 15

GIu Thr VaI Ala GIu Met Leu Ser GIu Ala
20 25

<210> 115

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 115

GIu Met Leu Ser GIu Ala GIu GIu Ala Ala GIu Lys Ala Ala GIu GIn
1 5 10 15

VaI GIu Ala Ala Lys GIu Ala VaI Ala GIu
20 25

<210> 116

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 116

Lys GIu Ala VaI Ala GIu Thr VaI GIy GIu Ala VaI GIy GIn VaI GIn
1 5 10 15

GIu Ala VaI Ala Thr Thr GIu
20

<210> 117

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 117

Lys Thr Tyr Thr Asn Ala Ser Ala Pro Pro Thr Ser lie lie Arg Lys
1 5 10 15

Cys Pro Lys Asn Ser Lys
20

<210> 118

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 118

Pro Lys Asn Ser Lys Thr Pro Thr Ser Ser Ser Ala Ser Thr Ala Cys
1 5 10 15

Ser Lys Cys Arg Asp Lys
20

<210> 119

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 119

Lys Cys Arg Asp Lys Pro Ala Ala Thr Pro Pro Lys Pro Cys Ala GIn
1 5 10 15

Ala Lys Thr Thr Phe Thr Ala Asn Ala
20 25

<210> 120

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 120

Pro Lys Asn Ser Lys Thr Pro Thr Ser Ser Ser Ala Ser Thr Ala Cys
1 5 10 15

Ser Lys Cys Arg Asp Lys Pro Ala Ala
20 25

<210> 121

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 121

Asp Lys Pro Ala Ala Thr Pro Pro Lys Pro Cys Ala Gln Ala Lys Thr
1 5 10 15

Thr Phe Thr Ala Asn Ala Ser Ala
20

<210> 122

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 122

Gln Val Lys Tyr Gln Gly Tyr Ile Asp Arg Gln Asn Glu Glu H e Asp
1 5 10 15

Ser Arg Arg Asp Ile Glu
20

<210> 123

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 123

Ser Arg Arg Asp H e Glu Thr Leu Lys Leu Pro Asp GIy H e Asp Tyr
 1 5 10 15

GIy Lys VaI Lys GIy Leu Ser Ala
 20

<210> 124

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 124

VaI Lys GIy Leu Ser Ala GIu VaI GIn GIn Lys Leu Asn GIn His Lys
 1 5 10 15

Pro GIu Thr VaI GIy GIn Ala Ser
 20

<210> 125

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 125

Lys Asn Ser Pro GIu Phe Asp Thr Leu Ser Pro Ala GIn Lys Thr Lys
 1 5 10 15

Leu Asn His Asp Leu Arg Asp Phe
 20

<210> 126

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 126

Arg	Arg	VaI	Leu	Pro	Leu	GIu	Asn	GIu	Ala	lie	Asn	GIu	Cys	Trp	Leu
1				5					10					15	

Ser	Asp	Arg	Asp	Arg	Phe	Ala	Tyr	GIu	GIy
			20					25	

<210> 127

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 127

Phe	Ala	Tyr	GIu	GIy	Leu	Tyr	His	GIu	Ser	Arg	Leu	Lys	Asn	Pro	Lys
1				5					10					15	

lie	Lys	GIn	GIy	GIy	GIu	Trp	Met	Asp	VaI
		20						25	

<210> 128

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 128

GIu	Trp	Met	Asp	VaI	Asp	Trp	Lys	Thr	Ala	Leu	GIu	Tyr	VaI	Arg	Ser
1				5					10					15	

Ala	lie	GIu	Cys	lie	Ala	Lys	Asp	GIy	Lys
			20					25	

<210> 129

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 129

His	Asp	GIy	His	GIy	Asp	Asp	Asp	His	GIy	His	Ala	Ala	His	GIn	His
1				5					10					15	

Asn	Lys	GIn	Asp	Lys	lie	lie	Ser	Arg
			20					25

<210> 130

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 130

Thr	Leu	Ser	Arg	Ser	GIy	lie	Arg	Thr	Asp	Phe	GIy	GIn	Pro	Pro	Arg
1				5					10					15	

Arg	Phe	Asp	Cys	Arg	Arg	Arg
			20			

<210> 131

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 131

Ala Asp Cys Ala Pro Thr GIy GIy Asn Arg lie Cys Pro Ala Arg Leu
 1 5 10 15

Ala Arg Phe Pro GIu
 20

<210> 132

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 132

Ser GIn Thr Pro Ala Pro GIn GIn Pro GIn Pro GIn Pro Asp Arg Ser
 1 5 10 15

Arg GIy Arg lie Leu
 20

<210> 133

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 133

Asn Gly Thr Arg Pro Ala H e His Arg Arg Thr Asp Met Ala Val Ala
 1 5 10 15

His Leu His Pro Pro Asp Ala GIy Lys
 20 25

<210> 134

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 134

Trp Leu Ala GIu GIn Ala GIu Asn Met GIy VaI GIu H e Tyr Pro GIy
 1 5 10 15

Phe Ala Ala Ala GIu VaI Leu Tyr His GIu Asp
 20 25

<210> 135

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 135

Val Leu Tyr His GIu Asp Gly Ser Val Lys Gly H e Ala Thr Gly Asn
 1 5 10 15

Met Gly H e Gly Lys Asp Gly
 20

<210> 136

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 136

Gly H e Gly Lys Asp Gly GIu Pro Thr Asp Ser Phe GIn Pro Gly Met
 1 5 10 15

GIu Leu Trp Ala GIn GIn
20

<210> 137

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 137

GIy Arg lie H e GIu Phe Thr GIn Asp Lys Arg Phe Asp Ser Arg Phe
1 5 10 15

Lys Asp Leu Lys Lys GIu Ser Leu Tyr Tyr
20 25

<210> 138

<211> 19

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 138

H e Val Leu Pro Val His Leu Pro Arg Cys Val Gly Cys Arg Arg Asp
1 5 10 15

Asp His GIn

<210> 139

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 139

VaI Leu Pro VaI His Leu Pro Arg Cys VaI GIy Cys Arg Arg Asp Asp
1 5 10 15

His GIn Arg Arg Asn GIu Thr Arg Leu
20 25

<210> 140

<211> 30

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 140

Lys Arg Arg GIy His Arg Ser VaI GIn GIn Asn Asn VaI GIu lie Ala
1 5 10 15

GIn Ala GIn Ala GIu Leu Ala Arg Arg GIn GIn GIu GIu Arg
20 25 30

<210> 141

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 141

GIu Asn GIy GIn Thr GIu Asn Asn Asp Trp lie Leu Met GIy Ser Thr
1 5 10 15

GIn GIu GIu Ala Lys Lys Asn Ala Met
20 25

<210> 142

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 142

Asn Asp Tyr GIu GIu lie Pro Leu Asp Ala Leu GIu Asp GIu Asp VaI
1 5 10 15

Ser GIu Ser VaI Asp Thr Ser Asp Lys GIn
20 25

<210> 143

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 143

Asp Thr Ser Asp Lys GIn Pro GIn Asp Asn Thr GIu Leu His GIu Lys
1 5 10 15

VaI GIu Thr VaI Ser Leu GIn Pro Arg Ala
20 25

<210> 144

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 144

Arg Lys His Asp Thr Pro His Gln His Pro Ser His Tyr Arg Arg Ser
1 5 10 15

Asp Ser Leu Leu Thr Val Cys Pro Gly Arg
20 25

<210> 145

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 145

Glu Thr Ala Arg Ala He Ala Glu Lys Glu Gly He Leu Val Gly He
1 5 10 15

Ser Ser Gly Ala Ala Val Trp
20

<210> 146

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 146

Arg He Trp Lys Lys Lys Thr Thr Lys Ala Ala Ala Asp Met Pro Cys
1 5 10 15

Ala He Cys Arg Thr Ala
20

<210> 147

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 147

lie Cys Arg Thr Ala GIy Leu Ser Arg Ser GIy Asn lie Ser VaI His
1 5 10 15

Asn H e Asp Thr Ala
20

<210> 148

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 148

Ala Ala Thr GIn Asn Asn Ala Ala Met Pro Ala Ala Ala Lys Leu Ala
1 5 10 15

Ala GIu Thr GIy VaI Asp
20

<210> 149

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 149

Ala GIu Thr GIy VaI Asp VaI Asn Ala Leu GIn GIy Ser GIy Arg Asp
1 5 10 15

GIy Arg VaI Leu Lys
20

<210> 150

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 150

VaI	GIu	Ala	GIu	His	Asn	Arg	Leu	Phe	Asp	Lys	Phe	His	Ala	GIn	GIu
1				5					10					15	

GIu	Ala	Trp	lie	Arg	Lys
			20		

<210> 151

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 151

Ser	Pro	His	Leu	Pro	Leu	VaI	Asn	Trp	Lys	Ser	Trp	Ala	lie	Ser	Pro
1				5					10					15	

GIn	Asp	Thr	Ala	Thr	Lys	Ser	Ser
				20			

<210> 152

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 152

Ala Thr Lys Ser Ser Pro Ala Thr Ser lie Leu Pro Asn Pro Pro Ser
1 5 10 15

Lys Ser Thr Phe Lys Thr Cys Ser
20

<210> 153

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 153

Phe Lys Thr Cys Ser Ala Asn Ser Thr Ser Ala Ala GIy Cys Arg Pro
1 5 10 15

Pro Phe Thr Pro Ser GIy Thr Thr Ser Pro
20 25

<210> 154

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 154

Phe Lys Thr Cys Ser Ala Asn Ser Thr Ser Ala Ala GIy Cys Arg Pro
1 5 10 15

Pro Phe Thr Pro Ser GIy Thr Thr
20

<210> 155

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 155

Lys	GIy	lie	Cys	Ser	Ala	Lys	GIn	GIy	GIn	Phe	GIy	Leu	Ala	GIu	Ala
1				5				10						15	

Tyr	Leu	Lys	Arg	Ser	Leu	Ala	Ala	GIn
			20					25

<210> 156

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 156

Pro	Lys	GIy	Lys	GIy	GIy	Trp	GIy	Asp	Arg	Lys	Ser	GIy	GIy	Trp	Arg
1				5				10						15	

GIy	Asp	His	Lys	Pro	Ser	Lys	GIu
			20				

<210> 157

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 157

Lys	Pro	Ser	Lys	GIu	GIy	Phe	GIy	GIy	Lys	Thr	Arg	GIy	GIu	GIy	Phe
1				5					10					15	

Lys Lys GIu GIy Phe Lys Arg Asp
20

<210> 158

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 158

GIy Phe Lys Arg Asp GIy Phe Lys Lys Thr GIy GIu GIy Phe Lys GIy
1 5 10 15

Lys Arg Lys Ala GIy Asp Ser Phe
20

<210> 159

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 159

Leu Pro Pro Thr Thr Ala Pro Pro Tyr Ser Ala Asp Ala lie Ser Ala
1 5 10 15

Thr Asn Thr Ser Lys Ser VaI Arg Thr Pro Phe
20 25

<210> 160

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 160

VaI Arg Thr Pro Phe Ser Pro lie Trp Thr Ser Ser Pro Pro Ala Ala
 1 5 10 15

Ser Ser Ala Lys Tyr Arg Thr Thr Ser Thr Ala
 20 25

<210> 161

<211> 29

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 161

Thr Thr Ser Thr Ala Thr GIy GIn Ala H e Pro Pro Thr Thr Pro Arg
 1 5 10 15

Ala Ser Ser Ala Ala Ala Thr Ser Ala Arg VaI Phe Lys
 20 25

<210> 162

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 162

Ala Ala Cys Thr Thr Asn **Pro** Leu **Pro** Pro Thr Thr **Ala** Pro **Pro** Tyr
 1 5 10 15

Ser Ala Asp Ala **H e** Ser Ala
 20

<210> 163

<211> 22

<212> PRT

<213> Artificial sequence

.

<220>

<223> Peptide fragment

<400> 163

Asp	Ala	lie	Ser	Ala	Thr	Asn	Thr	Ser	Lys	Ser	VaI	Arg	Thr	Pro	Phe
1				5					10					15	

Ser	Pro	lie	Trp	Thr	Ser
			20		

<210> 164

<211> 18

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 164

Pro	lie	Trp	Thr	Ser	Ser	Pro	Pro	Ala	Ala	Ser	Ser	Ala	Lys	Tyr	Arg
1				5					10					15	

Thr Thr

<210> 165

<211> 25

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 165

GIy Thr H e Phe Ala Ser Arg Pro Lys Thr GIn GIn Pro Phe GIn Thr
1 5 10 15

Ala Ser Lys Ser VaI H e Pro Thr Arg
20 25

<210> 166

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 166

Pro His Ala Thr Ser Lys Asn Thr Met Ser Lys Pro Ser Ala Asn Ser
1 5 10 15

Ser Asp Lys Pro Asn H e Pro Ser
20

<210> 167

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 167

Pro Thr Arg GIn Ser Ala Lys Ser Ala Pro Thr Ser Ala Ser Lys Thr
1 5 10 15

Pro Thr Pro Cys Ala Lys Lys Ala
20

<210> 168

<211> 27

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 168

Tyr	GIy	lie	Ser	Leu	Asn	Thr	Ala	Arg	Ser	Asp	Leu	Ser	Lys	Leu	GIy
1				5					10					15	

GIu	Tyr	Arg	Phe	Leu	VaI	Pro	Phe	Lys	Ser	GIy
			20					25		

<210> 169

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 169

Leu	Asn	Asn	GIn	GIy	Lys	Leu	Ser	GIn	Thr	GIy	Ser	GIn	Lys	Leu	His
1				5					10					15	

lie	Asp	Ala	GIn	GIy	Lys	Met	Asp	Asn	Arg
			20					25	

<210> n o

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 170

GIy	Lys	Met	Asp	Asn	Arg	GIy	Arg	Met	GIy	Leu	GIn	Asp	Thr	Ala	Pro
1				5					10					15	

Thr	Ala	Ser	Asn	GIy	Ser	Ser
			20			

<210> 171

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 171

Asn Met Leu Ala Ser GIy Leu Ala Ala Pro Thr GIn Ser GIy Ala GIy
1 5 10 15

lie Ala Ala Ala Thr Ala Ser Pro Ala VaI
20 25

<210> 172

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 172

GIy GIn Asn Ala Asn GIy Lys Leu Thr Ala Ser GIn GIu Thr Ala His
1 5 10 15

VaI Leu Ala His Ala VaI Leu
20

<210> 173

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 173

Trp Gln Ala Thr lie Thr Arg GIu Phe GIy Lys Asp lie Ala Val Lys
 1 5 10 15

Val GIy Asn Ser His GIu Ser GIy GIu Lys
 20 25

<210> 174

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 174

His GIu Ser GIy GIu Lys lie Asn Tyr Ser lie Arg Arg Asn Leu Ser
 1 5 10 15

Leu Asp Lys Ala Asp GIu Met lie
 20

<210> 175

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 175

Val Leu lie Gly **His** Ser Glu Arg Ser Leu Tyr Phe Gly Glu **Lys Asn**
 1 5 10 15

Glu **H e Gln** Arg Arg Lys Met
 20

<210> 176

<211> 28

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 176

Thr	Ala	Trp	VaI	Arg	Arg	Trp	GIn	Lys	Cys	Pro	Ser	Phe	Thr	Lys	Leu
1				5					10					15	

Pro	Ala	His	Ala	Ala	Ala	Ala	Arg	Arg	Asn	Ala	Lys
			20					25			

<210> 177

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 177

GIu	Lys	Arg	Arg	Ala	Asp	Arg	Pro	Leu	Arg	Arg	GIn	Pro	Ala	GIn	Arg
1				5					10					15	

Arg	GIn	Ala	GIy	Ser	VaI
			20		

<210> 178

<211> 20

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 178

Thr	Thr	Asp	Ala	Arg	Asn	Leu	Ala	Ala	Ala	Leu	GIy	Ser	Pro	Phe	Asp
1				5					10					15	

GIy GIy Thr GIn
20

<210> 179

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 179

GIn Asn Asn Tyr Pro Asp Arg VaI Arg lie GIy Ala GIy Arg GIn Tyr
1 5 10 15

Trp Arg Ser Asp GIu Asp GIu Pro Asn Asn
20 25

<210> 180

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 180

Tyr Ser Trp Leu VaI GIy GIy Asn Thr Phe Ala GIn Asn GIy Ser GIy
1 5 10 15

GIy GIy Thr VaI Asn Leu
20

<210> 181

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 181

Leu	Ser	GIu	Asn	Leu	Asn	Phe	Thr	Leu	GIn	Asn	GIu	His	VaI	Asp	Ala
1				5					10					15	

GIy	Ala	Trp	Arg	Tyr	GIn	Leu	lie	Arg	Lys
			20					25	

<210> 182

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 182

GIn	GIu	Leu	Ser	Asp	Lys	Leu	GIy	Lys	Ala	GIu	Ala	Lys	Lys	GIn	Ala
1				5					10					15	

GIu	Lys	Asp	Asn	Ala	GIn	Ser
			20			

<210> 183

<211> 26

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 183

Ala	Asp	Thr	Asn	Asp	Leu	Thr	Leu	Phe	Glu	Thr	GIu	Leu	Asp	Thr	Tyr
1				5					10				15		

H e	GIu	Arg	Val	GIu	Met	Ala	GIu	Ser	GIu
			20					25	

<210> 184

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 184

GIu Met Ala GIu Ser GIu Leu Asp Lys Ala Arg GIn GIy GIy Asp Ala
1 5 10 15

GIn Ala VaI GIu Thr Ala Arg His
20

<210> 185

<211> 59

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 185

GIy Leu Tyr Arg Lys Lys Pro Lys Pro His Arg Pro Ser Phe Pro GIn
1 5 10 15

Lys Arg GIu Ser Lys Asn Ala Thr GIn GIn GIu Phe H e GIy Lys Asn
20 25 30

Arg Asn Pro Thr Ala VaI H e Pro Ala Lys Ala GIy H e GIn GIn Pro
35 40 45

Lys Asn His Arg Asn Leu Ser Ala Lys Thr GIu
50 55

<210> 186

<211> 35

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 186

VaI	Lys	His	GIIn	VaI	His	Ser	VaI	GIu	Thr	Lys	Thr	Thr	lie	VaI	Thr
1				5					10					15	

Ser	Lys	Pro	Thr	GIIn	GIy	Ala	Ala	GIIn	GIy	GIy	Pro	H e	H e	GIIn	Thr
			20					25					30		

Asp	Pro	Ser
		35

<210> 187

<211> 71

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 187

Ser	Ser	Ser	Leu	GIy	Leu	Ser	Ala	H e	Tyr	Asp	Phe	Lys	Leu	Asn	Asp
1				5					10					15	

Lys	Phe	Lys	Pro	Tyr	H e	GIy	Ala	Arg	VaI	Ala	Tyr	GIy	His	VaI	Lys
			20					25					30		

His	GIIn	VaI	His	Ser	VaI	GIu	Thr	Lys	Thr	Thr	H e	VaI	Thr	Ser	Lys
		35					40					45			

Pro	Thr	GIIn	GIy	Ala	Ala	GIIn	GIy	GIy	Pro	H e	H e	GIIn	Thr	Asp	Pro
	50					55					60				

Ser	Lys	Pro	Pro	Tyr	His	GIu
65					70	

<210> 188

<211> 58

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 188

GIy Arg Arg Phe Lys Ser Ser GIn Thr His GIn GIu Arg GIy His Ser
 1 5 10 15

Ser VaI GIy Arg Ala Pro Ala Leu GIn Ala GIy GIy His Arg Phe Asp
 20 25 30

Pro VaI Cys Leu His GIn Tyr Cys Thr Asn GIn Asn GIy Arg Met GIu
 35 40 45

GIn Asn Pro Phe Arg Ala Thr Ser His Leu
 50 55

<210> 189

<211> 80

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 189

VaI GIu Ser Ala VaI GIu Thr VaI Ser GIy Ala VaI GIu GIn VaI Lys
 1 5 10 15

GIu Thr VaI Ala GIu Met Leu Ser GIu Ala GIu GIu Ala Ala GIu Lys
 20 25 30

Ala Ala GIu GIn VaI GIu Ala Ala Lys GIu Ala VaI Ala GIu Thr VaI
 35 40 45

GIy GIu Ala VaI GIy GIn VaI GIn GIu Ala VaI Ala Thr Thr GIu GIu
 50 55 60

His Lys Leu GIy Trp Ala Ala Arg Leu Lys GIn GIy Leu Thr Lys Ser
 65 70 75 80

<210> 190

<211> 58

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 190

GIn Ser GIu VaI GIn Met GIy Leu VaI Ser Lys Leu GIn Asp GIu Leu
 1 5 10 15

H e VaI H e GIn Thr GIn Leu Asp GIn VaI Lys Ala VaI Thr Pro GIu
 20 25 30

Asn Pro GIn H e Pro GIy Leu GIn Ala Arg GIu GIn Ser Leu Arg Lys
 35 40 45

GIu H e Asp GIn GIn Leu Arg Ala H e Ser
 50 55

<210> 191

<211> 58

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 191

GIn VaI Lys Tyr GIn GIy Tyr H e Asp Arg GIn Asn GIu GIu H e Asp
 1 5 10 15

Ser Arg Arg Asp H e GIu Thr Leu Lys Leu Pro Asp GIy H e Asp Tyr
 20 25 30

GIy Lys VaI Lys GIy Leu Ser Ala GIu VaI GIn GIn Lys Leu Asn GIn
 35 40 45

His Lys Pro GIu Thr VaI GIy GIn Ala Ser
 50 55

<210> 192

<211> 91

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 192

Lys Asn Ser Pro GIu Phe Asp Thr Leu Ser Pro Ala GIn Lys Thr Lys
 1 5 10 15

Leu Asn His Asp Leu Arg Asp Phe VaI Leu Ser GIy Ala GIu Leu Pro
 20 25 30

Pro GIu GIn GIn Ala GIu Leu Ala Lys Leu GIn Thr GIu GIy Ala GIn
 35 40 45

Leu Ser Ala Lys Phe Ser GIn Asn VaI Leu Asp Ala Thr Asp Ala Phe
 50 55 60

GIy lie Tyr Phe Asp Asp Ala Ala Pro Leu Ala GIy lie Pro GIu Asp
 65 70 75 80

Ala Leu Ala Met Phe Ala Ala Ala Ala GIn Ser
 85 90

<210> 193

<211> 61

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 193

Leu Thr Ser Ala Ala Thr Ala Leu Leu Ser lie Ser Ala Pro Ala Leu
 1 5 10 15

Ala His His Asp GIy His GIy Asp Asp Asp His GIy His Ala Ala His
 20 25 30

Gln His Asn Lys Gln Asp Lys lie lie Ser Arg Ala Gln Ala Glu Lys
 35 40 45

Ala Ala Leu Ala Arg Val GIy GIy Lys H e Thr Asp H e
 50 55 60

<210> 194

<211> 59

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 194

Val Leu Ser Ser GIy Arg Leu Thr Val Arg Asn Leu GIy Arg Leu Lys
 1 5 10 15

Asn Gln Asn Asn Gly Thr H e Gln Ala Ala Arg Leu Asp Met Ser Thr
 20 25 30

GIy GIy Leu Asp Asn Thr GIy Asn H e Thr Gln Thr GIy Ser Gln Ala
 35 40 45

Leu Asp Leu Val Ser Ala GIy Lys Phe Asp Asn
 50 55

<210> 195

<211> 60

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 195

Asn Pro Val Ser Pro Thr Ala Pro Ala Lys Asn Tyr Ala Val Gly Arg
 1 5 10 15

H e Gln Thr Thr Gly Ala Phe Asp Asn Ala Gly Ser H e Asn Ala Gly
 20 25 30

GIy GIn lie Asp H e Ala Ala GIn Asn GIy Leu GIy Asn Ser GIy Ser
 35 40 45

Leu Asn Ala Ala Lys Leu Arg VaI Ser GIy Asp Ser
 50 55 60

<210> 196

<211> 65

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 196

Leu Asp Tyr Ala GIn Trp Arg GIn Ser Leu Ala Ala Ala Asp Lys Ala
 1 5 10 15

Thr Ala GIn His GIn Ser Leu GIn Ala GIn GIn Asp GIu Thr Ala Ala
 20 25 30

GIn VaI GIn Ala Leu Asn Asp GIu VaI His Ala Leu GIn Thr Ala GIu
 35 40 45

GIn Ser GIn GIn GIn Ala VaI His GIu Leu Ser Asn Lys Arg GIy VaI
 50 55 60

Leu
 65

<210> 197

<211> 63

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 197

VaI Thr Asn Ala Lys Asn Thr H e Tyr Ala Ala Lys Arg Leu H e Gly
 1 5 10 15

His Lys Phe GIu Asp Lys GIu VaI GIn Arg Asp H e GIu Ser Met Pro
 20 25 30

Phe GIu H e H e Lys Ala Asn Asn GIy Asp Ala Trp VaI Lys Ala GIn
 35 40 45

GIy Lys GIu Leu Ser Pro Pro GIn H e Ser Ala GIu VaI Leu Arg
 50 55 60

<210> 198

<211> 60

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 198

Trp Leu Ala GIu GIn Ala GIu Asn Met GIy VaI GIu H e Tyr Pro GIy
 1 5 10 15

Phe Ala Ala Ala GIu VaI Leu Tyr His GIu Asp GIy Ser VaI Lys GIy
 20 25 30

H e Ala Thr GIy Asn Met GIy H e GIy Lys Asp GIy GIu Pro Thr Asp
 35 40 45

Ser Phe GIn Pro GIy Met GIu Leu Trp Ala GIn GIn
 50 55 60

<210> 199

<211> 64

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 199

GIu Thr Ser Asn Met Ala Leu GIu Lys GIu Ser Leu Lys Leu Leu Pro

1				5					10					15			
Phe	Leu	GIy	Asn	Leu	VaI	GIu	Asp	Asn	GIu	Ser	lie	Ala	GIu	Lys	Asn		
			20					25					30				
GIy	GIu	Thr	VaI	Thr	Leu	Ser	Ala	Asp	GIy	Lys	H e	Pro	GIu	Asn	Thr		
		35					40					45					
Thr	H e	Leu	Ala	Asn	GIu	Ser	Tyr	Leu	Tyr	Arg	Ala	Phe	Asp	Asn	VaI		
	50					55					60						

<210>	200
<211>	26
<212>	PRT
<213>	Artificial sequence

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<220>
<223> Peptide fragment
<400> 200
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Lys VaI Asn Leu GIy Tyr Lys Asn GIy Asp GIu VaI Cys VaI Arg Ser
1 5 10 15

Asp Tyr Thr GIy Tyr VaI Thr Cys Asn Thr
20 25

<210>	201
<211>	42
<212>	PRT
<213>	Artificial sequence

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<220>
<223> Peptide fragment
<400> 201
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Thr 1	Leu	Pro	Ala 5	Pro	Pro	Ser	Pro	Ser	Phe 10	Asp	Ser	Ser	Ala	Tyr 15	Ala
Ala	Pro	Arg	Ala 20	Leu	His	Asn	Pro	Asp 25	Trp	Tyr	Glu	Asn	Asp 30	Tyr	Glu

GIu lie Pro Leu Asp Ala Leu GIu Asp GIu
 35 40

<210> 202

<211> 81

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 202

Ser Leu Ala Asn Asn His VaI Asp Leu GIy Ala Leu Arg Tyr Thr lie
 1 5 10 15

Lys Thr GIu Asn GIy lie Thr Arg Leu Tyr Asn Pro Tyr Ala GIu Asn
 20 25 30

Arg Arg Arg VaI Lys Pro Ala Pro Ser Pro Ala Thr Asn Thr Ala Ser
 35 40 45

GIn Ala GIn Lys Ala Thr GIn Thr Asp GIy Ala GIn lie Ala Lys Pro
 50 55 60

GIn Asn H e VaI VaI Ala Pro Pro Ser Pro GIn Ala Asn GIn Ala GIu
 65 70 75 80

GIu

<210> 203

<211> 85

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 203

Ser Leu lie Thr Asp Pro Lys Thr H e Ser H e His Tyr H e GIn Asn
 1 5 10 15

Asn Asp Asp Asp Ala GIY Tyr Tyr Tyr Tyr Arg Pro Arg Lys Pro
 20 25 30

lie Pro GIn GIY Lys Asp Leu Tyr Phe Lys Asn Tyr Arg Tyr Tyr Ala
 35 40 45

Leu Lys Ser GIY GIY Ser VaI Asn Ala Pro Met Pro GIU Asn GIY GIn
 50 55 60

Thr GIU Asn Asn Asp Trp lie Leu Met GIY Ser Thr GIn GIU GIU Ala
 65 70 75 80

Lys Lys Asn Ala Met
 85

<210> 204

<211> 109

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 204

Ser VaI Asp Thr Ser Asp Lys GIn Pro GIn Asp Asn Thr GIU Leu His
 1 5 10 15

GIU Lys Tyr GIU Asn Asp Tyr GIU GIU lie Pro Leu Asp Ala Leu GIU
 20 25 30

Asp GIU Asp VaI Ser GIU Ser VaI Asp Thr Ser Asp Lys GIn Pro GIn
 35 40 45

Asp Asn Thr GIU Leu His GIU Lys VaI GIU Thr VaI Ser Leu GIn Pro
 50 55 60

Arg Ala Ala GIn Pro Arg Ala GIn Ala Ala Thr GIn Leu GIn Ala GIn
 65 70 75 80

Ala Ala Ala GIn Ala Asp Ala VaI Ser Thr Asn Thr Asn Ser Ala Leu
 85 90 95

Ser Asp Ala Met Ala Ser Thr GIn Ser lie Leu Leu Asp
 100 105

<210> 205

<211> 68

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 205

GIu Ala Ser Arg GIy Lys GIy Lys lie GIy Thr Thr GIy Arg GIy lie
 1 5 10 15

GIy Pro Ala Tyr GIu Asp Lys Val Ala Arg Arg Ala lie Arg Ala Ala
 20 25 30

Asp Leu Leu His Pro GIu Lys Leu Arg GIu Lys Leu Asp Ala Val Leu
 35 40 45

Ala Tyr Tyr Asn Val Gln Leu Gln His Leu His Asn Ala GIu Pro Val
 50 55 60

Lys Ala GIu Asp
 65

<210> 206

<211> 68

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 206

Asn lie Cys Val GIy Tyr GIu Leu Pro Asp GIy GIy Lys Thr Asp lie
 1 5 10 15

Leu Pro Cys GIy Ser Asp Ala Val GIu Thr Cys Lys Pro H e Tyr GIu
 20 25 30

Thr Met Pro GIy Trp Arg GIu Ser Thr Phe GIy Val Lys Asp Tyr GIy

35

40

45

Ala Leu Pro GIu Asn Ala Lys Ala Tyr Leu Lys Arg lie GIu GIu VaI
 50 55 60

Cys GIy Ala Pro
 65

<210> 207

<211> 36

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 207

Thr Pro Arg Tyr Arg lie GIy VaI Ser GIy Asp Tyr VaI Arg GIy Arg
 1 5 10 15

Leu Lys Asn Leu Pro Ser Leu Pro GIy Arg GIu Asp Ala Tyr GIy Asn
 20 25 30

Arg Pro Phe lie
 35

<210> 208

<211> 62

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 208

Gly Lys Leu Val Thr Gly Lys Asp Lys Gly GIu Asn Gly Ser Ser Thr
 1 5 10 15

Asp Glu GIy Glu Gly Leu Val Thr Ala Lys GIu Val H e Asp Ala Val
 20 25 30

Asn Lys Ala GIy Trp Arg Met Lys Thr Thr Thr Ala Asn GIy GIn Thr
 35 40 45

GIy GIn Ala Asp Lys Phe GIu Thr VaI Thr Ser GIy Thr Asn
 50 55 60

<210> 209

<211> 66

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 209

Thr Ala GIy Thr Asn GIy Asp Thr Thr VaI His Leu Asn GIy lie GIy
 1 5 10 15

Ser Thr Leu Thr Asp Thr Leu Leu Asn Thr GIy Ala Thr Thr Asn VaI
 20 25 30

Thr Asn Asp Asn VaI Thr Asp Asp GIu Lys Lys Arg Ala Ala Ser VaI
 35 40 45

Lys Asp VaI Leu Asn Ala GIy Trp Asn lie Lys GIy VaI Lys Pro GIy
 50 55 60

Thr Thr
 65

<210> 210

<211> 74

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 210

Ala Ser GIn Asn Asp Leu Ala Arg Ala VaI Ala Thr Arg Asn Arg
 1 5 10 15

H e Arg GIu Leu Asn Ala GIu Leu Lys GIn GIy Thr GIy Phe Ala Asp
20 25 30

Lys Met GIy Lys H e GIy Arg Phe GIy Ala Ala Ala VaI Ala GIy GIy
35 40 45

Ala Ala Ala Tyr Thr VaI Leu Lys Pro Ala Met Asp Asn Arg Lys GIn
50 55 60

Leu Asp GIu Asn H e Asn Arg VaI Ser Arg
65 70

<210> 211

<211> 42

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 211

VaI GIu Ala GIy Asn Lys Lys Met Asn Ala Ala Pro GIy Ala His Ala
1 5 10 15

His Leu GIy Leu Leu Leu Ser Arg Ser GIy Asp Lys GIu GIy Ala Phe
20 25 30

Arg GIn Phe GIu GIu GIu Lys Arg Leu Phe
35 40

<210> 212

<211> 30

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 212

Ala His Leu GIy Leu Leu Leu Ser Arg Ser GIy Asp Lys GIu GIy Ala
1 5 10 15

Phe Arg GIn Phe GIu GIu GIu Lys Arg Leu Phe Pro GIu Ser
 20 25 30

<210> 213

<211> 66

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 213

VaI Trp Phe Ser GIn Asn Pro lie Asn Ala Tyr Trp GIn GIn Thr Tyr
 1 5 10 15

His Arg Asn Ser Pro Leu GIu Pro Leu Ala Ala Tyr GIy Trp Trp Arg
 20 25 30

Ser GIy Ala Ala Leu GIn GIu Asn Ala Tyr Ala Leu Ser Asp GIy lie
 35 40 45

Lys Ala Phe Leu Ser GIy GIu Thr Pro Pro Thr Ala GIn Asp GIy GIy
 50 55 60

Ser Ala
 65

<210> 214

<211> 30

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 214

H e GIy Arg Thr GIy Arg Ala GIy Ala Asp GIy VaI Ala H e Ser Leu
 1 5 10 15

Met Asp Glu Ser GIu GIn Lys Met Phe GIu Ser H e Lys GIu

20

25

30

<210> 215

<211> 83

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 215

Ala Val Pro Ala Ala Ala Gln Pro Pro Val Gln Ser Ala Pro Val Asp
 1 5 10 15

H e Asn Ala Ala Thr His Thr lie Val Arg GIy Asp Thr Val Tyr Asn
 20 25 30

lie Ser Lys Arg Tyr His H e Ser Gln Asp Asp Phe Arg Ala Trp Asn
 35 40 45

GIy Met Thr Asp Asn Met Leu Ser lie GIy Gln H e Val Lys Val Lys
 50 55 60

Pro Ala GIy Tyr Ala Ala Pro Lys Thr Ala Ala Val GIu Ser Arg Pro
 65 70 75 80

Ala Val Pro

<210> 216

<211> 58

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 216

Thr Tyr Val Pro Ser Tyr Ala Pro Val Asp H e Asn Ala Ala Thr His
 1 5 10 15

Thr lie Val Arg GIY Asp Thr Val Tyr Asn lie Ser Lys Arg Tyr His
 20 25 30

lie Ser GIn Asp Asp Phe Arg Ala Trp Asn GIY Met Thr Asp Asn Thr
 35 40 45

Leu Ser lie GIY GIn H e Val Lys Val Lys
 50 55

<210> 217

<211> 58

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 217

GIY GIU Lys Ala Ala GIU Ala Pro Ala Ala GIU Ala Pro Ala Ala GIU
 1 5 10 15

Ala Pro Ala Thr GIU Ala Pro Ala Ala GIU Ala Pro Ala Ala GIU Ala
 20 25 30

Pro Ala Ala GIU Ala Pro Ala Ala GIU Ala Ala Ala Thr GIU Ala Pro
 35 40 45

Ala Ala GIU Ala Ala Ala Thr GIU Ala Pro
 50 55

<210> 218

<211> 64

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 218

Leu Ala Leu H e Ser Ala Ala Val H e Gly Leu Ala Ala Cys Ser GIn
 1 5 10 15

GIu Pro Ala Ala Pro Ala Ala GIu Ala Thr Pro Ala Ala GIu Ala Pro
 20 25 30

Ala Ser GIu Ala Pro Ala Ala GIu Ala Ala Pro Ala Asp Ala Ala GIu
 35 40 45

Ala Pro Ala Ala GIy Asn Cys Ala Ala Thr VaI GIu Ser Asn Asp Asn
 50 55 60

<210> 219

<211> 68

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 219

Ala Pro Ala Ser GIu Ala Pro Ala Ala GIu Ala Ala Pro Ala Asp Ala
 1 5 10 15

Ala GIu Ala Pro Ala Ala GIy Asn Cys Ala Ala Thr VaI GIu Ser Asn
 20 25 30

Asp Asn Met GIn Phe Asn Thr Lys Asp lie GIn VaI Ser Lys Ala Cys
 35 40 45

Lys GIu Phe Thr lie Thr Leu Lys His Thr GIy Thr GIn Pro Lys Ala
 50 55 60

Ser Met GIy His
 65

<210> 220

<211> 51

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 220

Pro Lys Pro Val GIu Pro Pro Lys Pro Val Leu Lys Pro Val Val Thr
 1 5 10 15

Lys Lys Ala Asp Ala Asp lie GIn GIn Pro Lys GIu GIu Pro Lys Pro
 20 25 30

GIu GIu Lys Pro Lys Pro GIu GIu Lys Pro Lys Pro GIu Pro Lys Pro
 35 40 45

GIu Ala Lys
 50

<210> 221

<211> 53

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 221

Pro Lys Pro GIu GIu Lys Pro Lys Pro GIu GIu Lys Pro Lys Pro GIu
 1 5 10 15

Pro Lys Pro GIu Ala Lys Pro Val Pro Lys Pro Ala GIu Lys Pro Val
 20 25 30

GIu Lys Pro Ser GIu Lys Pro Ala GIu His Pro GIy Asn Ala Ser Ala
 35 40 45

Lys Ala Asp Ser GIu
 50

<210> 222

<211> 37

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 222

Thr Val Lys Asn Leu Met Val Ala Val Ala Thr Ala GIy Val Ala Asp
 1 5 10 15

Lys lie GIy Ala Ser Ala Leu Asn Asn Val Ser Asp Lys GIn Trp lie
 20 25 30

Asn Asn Leu Thr Val
 35

<210> 223

<211> 60

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 223

Leu Asn Asn Arg H e Leu Thr Cys Ala GIn Asp Val Ser Arg Phe Pro
 1 5 10 15

Asn Asn GIy Ala Tyr Thr GIy GIu Val Ser Ala GIu Met Leu Ala Asp
 20 25 30

Thr GIy Thr Asp H e Val Leu H e GIy His Ser GIu Arg Ser Leu Tyr
 35 40 45

Phe GIy GIu Lys Asn GIu H e GIn Arg Arg Lys Met
 50 55 60

<210> 224

<211> 23

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 224

Thr GIy GIy Ser Phe GIy Asp Ser GIy Ser Pro Met Phe H e Tyr Asp
 1 5 10 15

Ala GIn Lys GIn Lys Trp Leu
 20

<210> 225

<211> 21

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 225

GIu Ala Lys Lys GIn Ala GIu Lys Asp Asn Ala GIn Ser Leu Asp Ala
 1 5 10 15

Leu lie Ala Ala GIy
 20

<210> 226

<211> 41

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 226

His Tyr Arg Ser GIn Asp Phe Arg Ala Tyr Arg GIn GIn Thr Asp Leu
 1 5 10 15

Arg GIn H e GIy Met GIn Lys Asn Leu GIy Ser GIy Arg VaI GIy H e
 20 25 30

Leu Phe Ser His Asn Arg Thr GIu Asn
 35 40

<210> 227

<211> 61

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 227

Ala Asp Thr Asn Asp Leu Thr Leu Phe GIu Thr GIu Leu Asp Thr Tyr
 1 5 10 15

lie GIu Arg VaI GIu Met Ala GIu Ser GIu Leu Asp Lys Ala Arg GIn
 20 25 30

GIy GIy Asp Ala GIn Ala VaI GIu Thr Ala Arg His Ala Tyr Leu Asn
 35 40 45

Ala Leu Asn Arg Leu Ser Arg GIn lie His Ser Leu Lys
 50 55 60

<210> 228

<211> 99

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 228

Asp GIn Ala Ala Thr Leu Thr Leu Thr GIy Asn Pro VaI Leu Ser Pro
 1 5 10 15

GIu His VaI GIu Trp VaI GIn Trp GIy Asn Arg Pro GIn GIy Asn Ala
 20 25 30

Ala VaI Tyr GIu Tyr lie Asn Pro His Arg Asn Arg Arg Thr Asp Tyr
 35 40 45

Phe lie Leu Lys Pro GIy GIy Asn Pro Arg GIu Phe Phe Pro Leu Asn
 50 55 60

Met Lys Asn Ser Thr Ser Trp GIn Phe H e GIy Asn Asn Arg GIn GIn
 65 70 75 80

Ala Ala GIu GIn VaI Ala GIn Ala GIu Asn Ala Arg Pro Asp Leu H e
 85 90 95

Thr Phe GIy

<210> 229

<211> 64

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 229

GIn Thr Leu Ser Lys Asp Arg His Phe Leu Arg Ser Ala Phe Lys Asn
 1 5 10 15

Pro Asn Lys Tyr GIy GIy Leu Ser Lys lie GIu GIu Lys Tyr Arg Lys
 20 25 30

Ser His GIu lie Phe Leu Lys Arg Leu Ala Ala Leu Pro Lys Pro GIu
 35 40 45

Phe Asp Asn Thr Leu Pro VaI His GIu Lys Leu GIu GIu H e Lys Lys
 50 55 60

<210> 230

<211> 76

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 230

GIn Asp GIy GIu Thr VaI VaI Ala Asp GIn VaI Leu Ala Arg VaI Asp
 1 5 10 15

Thr Ala Ala Thr Ala Ala Ala GIu Ala Pro Ala Ala Ala Pro Ala GIu

20

25

30

Ala Ala Pro Ala Ala Ala Pro Ala Ala Thr GIn Asn Asn Ala Ala Met
 35 40 45

Pro Ala Ala Ala Lys Leu Ala Ala GIu Thr GIy VaI Asp VaI Asn Ala
 50 55 60

Leu GIn GIy Ser GIy Arg Asp GIy Arg VaI Leu Lys
 65 70 75

<210> 231

<211> 59

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 231

Arg Phe Leu Asp Asn lie Ala Thr Arg lie VaI GIu Leu Asp Arg GIy
 1 5 10 15

lie Leu Arg Ser Tyr Pro GIy Ser Phe Ser Lys Tyr Ser GIu Lys Lys
 20 25 30

Ala GIn GIu Leu Ala VaI GIu Ala GIu His Asn Arg Leu Phe Asp Lys
 35 40 45

Phe His Ala GIn GIu GIu Ala Trp lie Arg Lys
 50 55

<210> 232

<211> 72

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 232

Tyr Phe Asp Lys Ala Leu Ala Asp Pro Thr Tyr Pro Thr Pro Tyr lie
1 5 10 15

Ala Asn Leu Asn Lys GIY H e Cys Ser Ala Lys GIn GIY GIn Phe GIY
20 25 30

Leu Ala GIU Ala Tyr Leu Lys Arg Ser Leu Ala Ala GIn Pro GIn Phe
35 40 45

Pro Pro Ala Phe Lys GIU Leu Ala Arg Thr Lys Met Leu Ala GIY GIn
50 55 60

Leu GIY Asp Ala Asp Tyr Tyr Phe
65 70

<210> 233

<211> 56

<212> PRT

<213> Artifxcial sequence

<220>

<223> Peptide fragment

<400> 233

Ser Lys Asp Leu Ala Arg Ser Met Ser Leu GIn Ser VaI Arg H e VaI
1 5 10 15

GIU Thr H e Ala GIY Lys Asn Thr Met GIY H e GIU Leu Pro Asn Asp
20 25 30

Lys Arg GIn Asp VaI Met Leu Ser GIU H e Leu Ser Ser Pro VaI Phe
35 40 45

Ala GIU Ala Lys Ser Lys Leu Thr
50 55

<210> 234

<211> 87

<212> PRT

<213> Artificial sequence

<220>

<223> Peptide fragment

<400> 234

Tyr Arg Asn Leu GIn Thr lie Pro Ser H e GIy Lys Asp Thr Ala Ser
1 5 10 15

VaI Leu Tyr Ala GIn Leu Thr GIu Lys His Phe Lys Thr Ala Asn GIn
20 25 30

Phe VaI Ser Tyr Ala GIy Leu Asn Pro Ala H e H e GIn Ser GIy Thr
35 40 45

Ser VaI Arg GIy Arg GIy Arg Leu Ser Arg Tyr GIy Asn Arg Arg Leu
50 55 60

Lys Ser Thr Leu Tyr Met Pro Ala Leu Cys Ala Tyr Arg Phe Asn Ala
65 70 75 80

Phe Pro Lys Leu H e Asn Asn
85