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(54) **Title**: IMMUNOGENIC COMPLEX FOR ELICITING PROTECTIVE IMMUNITY AGAINST GROUP B STREPTOCOCCUS

(57) **Abstract**: The present invention relates to an immunogenic complex comprising an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, and a capsular polysaccharide. The immunogenic complex is capable of eliciting protective immunity against group B Streptococcus. The invention further pertains to an immunogenic product comprising the immunogenic complex and an immunogenic fusion protein, the vaccine, the immunogenic complex,or the immunogenic product for use in a method of preventing or treating a group B Streptococcus infection, as well as a method of preventing or treating a group B Streptococcus infection.

IMMUNOGENIC COMPLEX FOR ELICITING PROTECTIVE IMMUNITY AGAINST GROUP B STREPTOCOCCUS

FIELD OF INVENTION

The present invention relates to the fields of microbiology and vaccine technology, and concerns an immunogenic complex comprising an N-terminal region of a group B Streptococcus surface protein and a capsular polysaccharide. The invention further pertains to an immunogenic product comprising the immunogenic complex and an immunogenic fusion protein, a vaccine comprising the immunogenic complex and/or immunogenic product, and a method of preventing or treating a group B Streptococcus infection.

BACKGROUND OF THE INVENTION

Group B Streptococcus (*Streptococcus agalactiae)* (GBS) is the major cause of invasive bacterial infections, including meningitis, in the neonatal period. In the United States alone, there are now about 5000 cases per year of invasive disease caused by this bacterium. These infections have an overall mortality of about 10%, and many of the infants that survive have permanent neurological sequelae. In view of this, a large effort has been made to find methods of prevention and treatment and to analyze the mechanisms by which GBS cause infections.

GBS can also cause mastitis in cows, a bovine disease that is of considerable economical importance. Development of a vaccine against GBS infections is therefore of interest also in veterinary medicine.

About 20 % of all women are vaginal carriers of GBS, and vertical transmission from the maternal genital tract is probably the most common source of infection in neonatal disease caused by this bacterium. However, only about 1 % of the infants that are colonized by the GBS at birth are afflicted by serious infection. Other factors than exposure to the bacterium during birth must therefore contribute to the development of neonatal disease.

Group B streptococcal strains are divided into nine serotypes (Ia, Ib, and II-VIII) based on the structure of the polysaccharide capsule (Baker, J Inf Dis 1990. 161: 917). The four “classical” serotypes Ia, Ib, II, and III occur in roughly equal proportions among strains in the normal flora, but type III is the clinically most important serotype, in particular because it causes most cases of meningitis. Because the capsule is a known virulence factor, it has been studied in considerable

detail, in particular in type III strains. Efforts have been made to develop a vaccine, in which the type III polysaccharide capsule would be an essential component.

EP 0 866 133 discloses a vaccine capable of protecting a recipient from infection caused by group B *Strepococcus*. The invention is directed to the use of a combination of a polysaccharide and a fragment of the epsilon protein. It further discloses that epidemiological data suggest that the type-specific capsule plays an important role in the immunity to group B *Streptococcus* infections (se page 7 line 2-3).

The document Gravekamp et al., Infection and Immunity, Dec 1997, p 5216-5221 discloses the evaluation of the immunogenicity as well as protection of the number of repeats of the alpha (α) C, i.e. AlpC, protein as well as the N-terminal part alone.

WO 9410317 describes the use of the alpha protein, a GBS surface protein, in the development of a conjugate vaccine. A drawback with this protein is that it usually is not expressed by type III strains, which are the cause of many serious GBS infections. Hence, a protective immunity against these strains will not be evoked by an alpha protein vaccine.

WO 9421685 describes the use of the Rib protein, a GBS surface protein, in the development of a vaccine. This protein elicits immunity when administered with alum. However, the Rib protein has the disadvantage that it does not evoke a protective immunity against all GBS strains.

WO 2008127179 describes a fusion protein comprising at least one first N-terminal region fragment of a group B Streptococcus surface protein or analogue, homologue, derivative or immunologically related amino acid sequence or fragments thereof, which is fused to at least one second N-terminal region fragment of a group B Streptococcus surface protein or analogue, homologue, derivative or immunologically related amino acid sequence or fragments thereof, wherein the first and second at least one N-terminal region fragments of group B Streptococcus surface proteins derive from different group B Streptococcus strains, and wherein the fusion protein is capable of eliciting protective immunity against group B Streptococcus.

The document Lindahl et al, Nonimmunodominant Regions Are Effective As Building Blocks In A Streptococcal Fusion Protein Vaccine, Cell Host & Microbe 2, 427-434, December 2007, discloses a fusion protein comprising N-terminal regions of the group B Streptococcus surface proteins Rib and AlpC.

Despite the advances in the progress towards a vaccine suitable for prevention of GBS disease, there is still a need for further methods and vaccines for prevention and treatment of GBS infections. Thus, there remains a need to explore vaccine strategies capable of eliciting protective immunity against a wide range of GBS stains.

Accordingly, it is a primary objective of the present invention to provide an immunogenic complex comprising an N-terminal region of a group B Streptococcus surface protein and a capsular polysaccharide which can be used in a vaccine capable of eliciting protective immunity against GBS infections.

It is a further objective of the present invention to provide a vaccine that elicits protective immunity against many clinically important GBS strains.

Another objective of the present invention is to provide a vaccine comprising a single, or a few, components that elicits protective immunity against GBS infections. A single or a few components has several advantages over a vaccine composed of numerous components, e.g. cost of production and safety.

The means of accomplishing each of the above objectives as well as others will become apparent from the description of the invention which follows hereafter.

SUMMARY OF THE INVENTION

The present invention is based on realization, by the present inventors, that the non-immunodominant N-terminal regions of group B Streptococcus surface proteins, of which the use of the N-terminal regions of the surface proteins Rib and AlpC in the form of a fusion protein is disclosed in WO 2008127179, despite their non-immunodominancy can still be useful on their own and not only in the form of a fusion protein. The way to realise this usefulness is to employ these N-terminal regions as carriers for a capsular polysaccharide. As discussed above capsular polysaccharides have been used in vaccines, however, according to EP 0 866 133 the type specific capsule plays a major role in the immunity, thus the width of protection against a range of different group B streptococcus strains is limited using capsular polysaccharides. By using an N-terminal region of a group B Streptococcus surface protein as a carrier for the capsular polysaccharide the immunogenicity and scope of protection will be increased.

Thus a first aspect of the present invention relates to an immunogenic complex comprising:

an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein,

and

a capsular polysaccharide,

wherein the immunogenic complex is capable of eliciting protective immunity against group B Streptococcus.

A major advantage of the immunogenic complex according to the first aspect of the present invention is that it represents a hybrid between earlier used capsular polysaccharides techniques and the more recent fusion protein techniques to thereby increase the scope of protection obtained. The group B Streptococcus surface protein may be selected from surface proteins which are expressed by many clinically important strains of group B Streptococcus, and will therefore give the immunogenic complex a wide scope of protection against these important strains. Further the hybrid nature of the immunogenic complex, comprising both an amino acid sequence and a capsular polysaccharide, will provide better immunogenicity than either of the amino acid sequence and the capsular polysaccharide on their own. Thus it is expected that the immunogenic complex will be immunogenic even without adjuvant, although it can also be used with an adjuvant such as alum or Aluminium hydroxide (AlOH).

A second aspect of the present invention pertains to an immunogenic product comprising the immunogenic complex according to the first aspect of the present invention, wherein the immunogenic product further comprises an immunogenic fusion protein comprising:

a first amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a first group B Streptococcus surface protein, which is fused to

a second amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a second group B Streptococcus surface protein

wherein each of the first and the second group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein and AlpC protein, and wherein the immunogenic fusion protein is capable of eliciting protective immunity against group B Streptococcus.

Thus another advantage with the present invention is it also pertains to a immunogenic product which comprises the immunogenic complex according to the first aspect of the present invention combined with an immunogenic fusion protein, such as for example the Rib-AlpC-NN fusion protein of WO 2008127179, thus providing an immunogenic product capable of providing full coverage of protection against all clinically relevant Group B Streptococcus strains using only one immunogenic complex and one immunogenic fusion protein.

The third aspect of the present invention pertains to a vaccine comprising a pharmaceutically acceptable vehicle, optionally an adjuvant, and a pharmaceutically effective amount of an immunogenic complex according to the first aspect of the present invention or an immunogenic product according to the second aspect of the present invention, wherein the vaccine is capable of eliciting protective immunity against group B Streptococcus.

The corresponding fourth and fifth aspect of the present invention pertain to the immunogenic complex according to the first aspect of the present invention, the immunogenic product according to the second aspect of the present invention, and/or the vaccine according to the third aspect of the present invention for use in a method of preventing or treating an infection caused by a group B Streptococcus, and
a method of preventing or treating an infection caused by a group B Streptococcus comprising administering to the immunogenic complex according to the first aspect of the present invention, the immunogenic product according to the second aspect of the present invention, and/or the vaccine according to the third aspect of the present invention,
respectively.

DETAILED DESCRIPTION OF THE INVENTION

In this specification, unless otherwise specified, “a” or “an” means “one or more”.

Throughout the specification, any and all references are specifically incorporated into this patent application by reference.

In a first embodiment of the immunogenic complex according to the first aspect of the present invention the immunogenic complex comprises:

an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, and

a capsular polysaccharide,

wherein the immunogenic complex is capable of eliciting protective immunity against group B Streptococcus.

The term “immunogenic” is intended to mean having the ability to elicit an immune response. The immunogenic complex of the invention is immunogenic and characterised by its ability to elicit a protective immune response against at least GBS expressing the surface protein of which the N-terminal region is comprised by,

or GBS expressing the capsular polysaccharide.
In the complex the amino acid sequence works as a carrier for the capsular polysaccharide. Thus the capsular polysaccharide may be covalently bound to the amino acid sequence

The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. If the two sequences to be compared are not of equal length, they must be aligned to best possible fit. Sequence identity can, for example, be calculated by the BLAST program e.g. the BLASTP program or the BLASTN program (Pearson W. R and D. J. Lipman (1988) PNAS USA 85:2444-2448) (www.ncbl.nlm.nlh.gov/BLAST).

The term “N-terminal region” in relation to the present invention refers to an N-terminus region (N) of a protein. Examples of amino acid sequences of the N-terminal regions of the group B Streptococcus surface proteins are given in SEQ IDs NO: 2, 4, 8, 10 and 14.
In particular, examples of N-terminal regions of group B Streptococcus proteins include the N-terminal region of the group B Streptococcus Rib, Alp1, Alp2, Alp3, Alp4 and AlpC protein, including peptides encoding native amino acid sequences of N-terminal regions of natural Rib, Alp1, Alp2, Alp3, Alp4 and AlpC protein.

Group B streptococcal strains, also referred herein as GBS, are well known and may be isolated from the blood of infected human beings. GBS is the most common cause of neonatal sepsis in the United States and is responsible for about 5000 cases per year.
The denotation “Group B streptococcal” and “Group B streptococcus” derives from the fact that Streptococci have been divided into immunological groups based upon the presence of specific carbohydrate antigens on their cell surfaces. At present, groups A through O are recognized (Davis, B.D. et al., In: Microbiology, 3rd. Edition, page 609, (Harper & Row, 1980).

The capsular polysaccharide is preferably a bacterial polysaccharide, more preferably a group B Streptococcus polysaccharide.
The capsular polysaccharide may be serotype specific and selected from group consisting of Group B Streptococcus serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII, IX and X.
By polysaccharide is meant any linear or branched polymer consisting of monosaccharide residues, usually linked by glycosidic linkages, and thus includes oligosaccharides. Preferably, the polysaccharide will contain between 2 and 50 monosaccharide unites, more preferably between 6 and 30 monosaccharide units.
The polysaccharide component may be based on or derived from polysaccharide

components of the polysaccharide capsule from many Gram positive and Gram negative bacterial pathogens such as *H. influenzae*, *N. meningitidis* and *S. pneumoniae*. Other bacteria from which polysaccharide components may be conjugated to the carrier proteins of the present invention include *Staphylococcus aureus*, *Klebsiella, Pseudomonas, Salmonella typhi, Pseudomonas aeruginosa*, and *Shigella dysenteriae*. Polysaccharide components suitable for use according to this aspect of the present invention include the Hib oligosaccharide, lipopolysaccharide from *Pseudomonas aeruginosa* (Seid and Sadoff, 1981), lipopolysaccharides from Salmonella (Konadu et al., 1996) and the O-specific polysaccharide from *Shigella dysenteriae* (Chu et al, 1991). Other polysaccharide components suitable for use in accordance with the present invention will be well-known to those skilled in the art. Fragments of bacterial capsular polysaccharide may be produced by any suitable method, such as by acid hydrolysis or ultrasonic irradiation (Szn et al, 1986). Other methods of preparation of the polysaccharide components will be well known to those of skill in the art.

Preferably, as stated above, the polysaccharide is a capsular polysaccharide derived from group B Streptococcus, or their equivalents.

The capsular polysaccharide should preferably be coupled to the amino acid sequence by a covalent linkage. A particularly preferred method of coupling polysaccharide and the amino acid sequence is by reductive amination. Other methods include: activation of the polysaccharide with cyanogen bromide followed by reaction with adipic acid dihydrazide (spacer) and by conjugation to carboxide groups of carrier amino acid sequences or protein using soluble carbodiimides (Shneerson et al, 1986); functionalisation of the carrier amino acid sequence or protein with adipic acid dihydrazide followed by coupling to cyanogen bromide activated polysaccharides (Dick et al, 1989); chemical modification of both the carrier amino acid sequence and the polysaccharide followed by their coupling (Marburg et at, 1986; Marburg et al, 1987 and 1989).

The polysaccharide molecule may be coupled to the amino acid sequence by a spacer molecule, such as adipic acid. This spacer molecule can be used to facilitate the coupling of amino acid sequence to polysaccharide. After the coupling reaction has been performed, the immunogenic complex or conjugate may be purified by diafiltration or other known methods to remove unreacted amino acid sequence or polysaccharide components.

If the polysaccharide is derived from a bacterial pathogen different from GBS, the conjugate may elicit immunity against two or more pathogens, e.g. multiple types of bacteria. This is a potentially important application of the immunogenic complex.

Multiple capsular polysaccharides may be coupled to the same amino acid sequence. Thus the immunogenic complex may comprise multiple capsular polysaccharides each linked to the amino acid sequence by any of the techniques

and/or linkers described above. Where the immunogenic complex comprises multiple capsular polysaccharides the capsular polysaccharides may be identical or different. When the capsular polysaccharides are different they may be derived from different bacteria, e.g. from different Group B Streptococcus serotypes.
The number of capsular polysaccharides in the immunogenic complex may thus be one or more such as 1, 2, 3, or more.

The term "protective immunity" in relation to the present invention refers to the ability of serum antibodies and/or cytotoxic T cell response induced during immunization to protect (partially or totally) against disease caused by an infectious agent, such as a group B Streptococcus. That is, a vertebrate immunized by the vaccines of the invention will experience limited growth and spread of group B Streptococcus. To determine whether protective immunity is induced by a immunogenic complex or vaccine, techniques well known for a person skilled in the art can be used. For example, to determine whether immunization with an immunogenic complex or vaccine according to the invention induces protective immunity against group B Streptococcus infection, immunized test animals can be challenged with group B Streptococcus and growth and spread of the group B Streptococcus is measured.

In the preferred embodiment of the immunogenic complex according to the first aspect of the present invention the group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein and AlpC protein.
This is advantageous as these proteins are expressed by clinically relevant group B Streptococcus serotypes/strains.
The group B Streptococcus Rib protein, also referred to in this specification as Rib and Rib protein, is a surface protein known in the art, and for example described in WO 9421685. The denotation "Rib" refers to: Resistance to proteases, immunity, and group B. The Rib protein was first isolated from a group B streptococcal strain of serotype III as a distinct 95 kDa protein. Protein Rib is expressed by almost all group B streptococcal strains of the clinically important serotype III, which cause most cases of meningitis, and by some strains of other serotypes such as II. Moreover, Rib is expressed by all strains of a hypervirulent clone of type III. A method has been devised to purify protein Rib and it has been demonstrated that antibodies to this protein protect against lethal infection with strains expressing protein Rib (for further details, such as DNA and protein sequences see WO 9421685). The nucleic acid sequence and the amino acid sequence for the N-terminal region of Rib are given in SEQ ID Nos: 1 and 2.

The Alp1 protein is also known as epsilon protein and is a group B streptococcal

alpha-protein-like protein (Creti et al. *Clin Microbiol.* 2004.42:1326-9).
The nucleic acid sequence and the amino acid sequence for the N-terminal region of Alp1 are given in SEQ ID Nos: 7 and 8.

The Alp2 protein is another alpha-protein-like-protein first identified in a serotype V strain (Lachenauer, C. S., R. Creti, J. L. Michel, and L. C. Madoff. 2000. Mosa-icism in the alpha-like protein genes of group B streptococci. Proc. Natl. Acad. Sci. USA 97:9630–9635.). Like the other members of the family, the Alp2 protein has an N-terminal domain and several repeated domains towards the C-terminus. Subsequently that protein has been found also in other GBS isolates such as serotypes Ia and III (Lindahl et al. Surface Proteins of Streptococcus agalactiae and Related Proteins in Other Bacterial Pathogens, CLINICAL MICROBIOLOGY REVIEWS, Jan. 2005, p. 102–127). The nucleic acid sequence and the amino acid sequence for the N-terminal region of Alp2 are given in SEQ ID Nos: 9 and 10.

The Alp3 protein is yet another alpha-protein-like-protein, also know as R28. It is very similar to the R28 protein also found in S. pyrogenes. (Lachenauer, C. S., R. Creti, J. L. Michel, and L. C. Madoff. 2000. Mosa- icism in the alpha-like protein genes of group B streptococci. Proc. Natl. Acad. Sci. USA 97:9630–9635 and Lindahl et al. Surface Proteins of Streptococcus agalactiae and Related Proteins in Other Bacterial Pathogens, CLINICAL MICROBIOLOGY REVIEWS, Jan. 2005, p. 102–127). The structure is more complex than the other Alpha-protein-like-proteins, but it retains an N-terminal domain which is identical to that of Alp2, and C-terminal repeat regions very similar to Rib. The nucleic acid sequence and the amino acid sequence for the N-terminal region of Alp3 are the same as for Alp2 and are given in SEQ ID Nos: 9 and 10.

The Alp4 protein is an alpha-protein-like-protein so far only identified in the Prague 25/60 strain (Fanrong Kong, Sonia Gowan, Diana Martin, Gregory James, and Gwendolyn L. Gilbert. Molecular Profiles of Group B Streptococcal Surface Protein Antigen Genes: Relationship to Molecular Serotypes. JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2002, p. 620–626). It is a novel member of the Alpha-protein-like family with a structure similar to that of the other members, with a distinct N-terminal domain, and repeat regions towards the C-terminus. The nucleic acid sequence and the amino acid sequence for the N-terminal region of Alp4 are given in SEQ ID Nos 13 and 14.

The group B Streptococcus AlpC protein, also known as alpha protein, is a group B Streptococcus surface protein known in the art. WO 9410317 describes a conjugate vaccine composition comprising the alpha protein. The native group B Streptococcus AlpC precursor protein as described in WO 9410317 has a molecular

weight of 108 kDa. Cleavage of the putative signal sequence of 41 amino acids yields a mature protein of 104 kDa. (Note, however, that the signal sequence was subsequently shown to have a length of 56 amino acid residues: Stålhammar-Carlemalm et al., J Exp Med 177,1593; 1993). The 20 kDa N-terminal region of the AlpC antigen shows no homology to previously described protein sequences and is followed by a series of nine tandem repeating units that make up 74% of the mature protein. Each repeating unit (denoted herein as "R") is identical and consists of 82 amino acids with a molecular mass of about 8500 Daltons, which is encoded by 246 nucleotides. The C-terminal region of the AlpC antigen contains a cell wall anchor domain motif present in a number of Gram-positive surface proteins.
The nucleic acid sequence and the amino acid sequence for the N-terminal region of AlpC are given in SEQ ID Nos: 3 and 4.

Each of the Rib, Alp1, and AlpC proteins of GBS includes a unique N-terminal region (N) and a long repeat (R) region. The proteins expressed by the GBS strains BM110 and A909 have 12 and 9 repeats, respectively. The wall anchoring regions are located at the C-terminal ends.
The N-terminal regions of Alp2 and Alp3 are identical.

The tandem repeats in Rib and alpha are identical within each protein, but not between the proteins, and vary in number between isolates. Except for this variation, the sequences of Rib and alpha are stable among strains. The two proteins show little or no antigenic cross-reactivity.

More preferably the group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp4 protein, such as the group consisting of Alp2 protein, Alp3 protein, and Alp4 protein.

In one embodiment of the immunogenic complex according to the first aspect of the present invention the immunogenic complex further comprises a further amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a further group B Streptococcus surface protein
This is advantageous as it provides for an immunogenic fusion protein capable of eliciting protective immunity against a larger number of group B Streptococcus strains. Thus the further amino acid sequence may be fused to the amino acid sequence to form a fusion protein.
In a further embodiment of the immunogenic complex according to the first aspect of the present invention the further group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp 4 protein and AlpC protein.
More preferably the further group B Streptococcus surface protein is selected from

the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp 4 protein, such as the group consisting of Alp2 protein, Alp3 protein, and Alp 4 protein.
Where the immunogenic complex comprises multiple capsular polysaccharides each of the capsular polysaccharides may be carried, by being covalently attached or attached via a linker, to a corresponding one of the amino acid sequences. Thus, where the immunogenic complex comprises two capsular polysaccharides and two amino acid sequences one of the capsular polysaccharides may be attached to one of the amino acid sequences and the other of the capsular polysaccharides may attached to the other one of the amino acid sequences.
In one embodiment of the immunogenic complex according to the first aspect of the present invention the group B Streptococcus surface protein and the further group B Streptococcus surface proteins are derived from different group B Streptococcus strains.
This will imply slight variability in the sequence of the N-terminal region fragments but would not alter the biological properties and their functional ability to elicit protective immunity. The group B Streptococcus surface protein and the further group B Streptococcus surface proteins may also be different. This is advantageous as it increases the number of group B Streptococcus strains which the immunogenic complex according to the first aspect of the present invention provides protection against.
In one preferred embodiment of the immunogenic complex according to the first aspect of the present invention there is only one amino acid sequence, having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, in the immunogenic complex.
This means that the immunogenic complex contains only one amino acid sequence having at least 80% sequence identity with the N-terminal region of a group B Streptococcus surface protein.
The group B Streptococcus surface protein may be selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein, and AlpC protein.

In a further embodiment of the immunogenic complex according to the first aspect of the present invention the group B Streptococcus surface protein and the capsular polysaccharide, and optionally also the further group B Streptococcus surface protein, are derived from different group B Streptococcus serotypes.
This is advantageous as it increases the number of group B Streptococcus serotypes which the immunogenic complex according to the first aspect of the present invention provides protection against.

In a further embodiment of the immunogenic complex according to the first aspect of the present invention the amino acid sequence has at least 80%, such as at least

85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14, and optionally the further amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14.

In the preferred embodiment of the immunogenic complex according to the first aspect of the present invention the amino acid sequence, and optionally also the further amino acid sequence, is conjugated to the capsular polysaccharide. Conjugation encompasses covalently attaching, either directly or via a linker structure or chain as described above. Where the immunogenic complex comprises multiple capsular polysaccharides each capsular polysaccharide may alternatively be conjugated to a single one of the amino acid sequences

In one embodiment of the immunogenic complex according to the first aspect of the present invention the amino acid sequence, and optionally also the further amino acid sequence, is modified by glycosylation, amidation, carboxylation or phosphorylation, or by being conjugated to an RSV antigen.
RSV antigens are described below with reference to the third aspect of the present invention.
This is advantageous as such polypeptides, i.e. amino acid sequences, may have enhanced immunogenicity. Such polypeptides may result when the native forms of the polypeptides or fragments thereof are modified or subjected to treatments to enhance their immunogenic character in the intended recipient. Numerous techniques are available and well known to those of skill in the art which may be used, without undue experimentation, to substantially increase the immunogenicity of the polypeptides herein disclosed. For example, the polypeptides may be modified by coupling to dinitrophenol groups or arsanilic acid, or by denaturation with heat and/or SDS. For a review of some general considerations in coupling strategies, see Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, ed. E. Harlow and D. Lane (1988). Useful immunogenic carriers are well known in the art. Examples of such carriers are keyhole limpet hemocyanin (KLH); albumins such as bovine serum albumin (BSA) and ovalbumin, PPD (purified protein derivative of tuberculin); red blood cells; tetanus toxoid; cholera toxoid; agarose beads; activated carbon; or bentonite.

A second aspect of the present invention concerns an immunogenic product comprising the immunogenic complex according to any of the preceding claims, wherein the immunogenic product further comprises and immunogenic fusion protein comprising:

a first amino acid sequence having at least 80% sequence identity with the

amino acid sequence of the N-terminal region of a first group B Streptococcus surface protein, which is fused to

a second amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a second group B Streptococcus surface protein

wherein each of the first and the second group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein and AlpC protein, and wherein the immunogenic fusion protein is capable of eliciting protective immunity against group B Streptococcus. This is advantageous as it provides an immunogenic product capable of providing full coverage of protection against all clinically relevant Group B Streptococcus strains using only one immunogenic complex and one immunogenic fusion protein.

In the preferred embodiment of the immunogenic product according to the second aspect of the present invention the first amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14, and wherein the second amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14,

or alternatively

the immunogenic fusion protein comprises an amino acid sequence having at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with any one of the amino acid sequences SEQ ID NO:6 and 12.

For the purpose of the present invention the term "fusion protein" refers to an assembly of two or more proteins or or regions of proteins, comprising for example an N-terminal region of a group B Streptococcus Alp1 protein and an N-terminal region of a group B Streptococcus Alp2 protein. For example there might be one N-terminal region of the Alp1- and one N-terminal region of the Alp2, or 2, 3, 4 or 5 N-terminal region fragments of the Alp1- and the Alp2-proteins, wherein the numbers of N-terminal regions from the two proteins need not be equal.

The combination of polypeptides to provide a fusion protein can be accomplished by coupling or conjugation, either either directly or through an intermediate structure, or by molecular biological fusion, i.e. through the combination of recombinant nucleic acid molecules which comprise fragments of nucleic acid capable of encoding each of the two, such that a single continuous expression product is finally produced.

For the purpose of the present invention the term "protein" refers to a molecular chain of amino acids. A protein is not of a specific length and can, if required, be modified in vivo or in vitro, by, for example, glycosylation, amidation, carboxylation or phosphorylation. Inter alia, amino acid sequences, peptides, oligopeptides and polypeptides are included within the definition. The protein or peptide can be of natural or synthetic origin. In this context a fusion protein is intended to mean two or more polypeptides covalently linked to each other either directly or indirectly by several means such as those mentioned above. The term “fused” means to create a fusion protein as mentioned above.

In addition to, or as replacement for, the immunogenic fusion protein the immunogenic product may comprise one or more further immunogenic complex according to the first aspect of the present invention. Thus the immunogenic product according to the second aspect of the present invention may for example comprise only two immunogenic complexes according to the first aspect of the present invention, only one immunogenic complex and one immunogenic fusion protein as discussed above, two or more immunogenic complexes according to the first aspect of the present invention and one immunogenic fusion protein as discussed above, or two or more immunogenic fusion complexes according to the first aspect of the present invention and two or more immunogenic fusion proteins as discussed above. In each case the amino acid sequence and capsular polysaccharide of the immunogenic complex or complexes may be derived from the same or different serotypes/strains, and the first and second amino acid sequences of the immunogenic fusion protein or fusion proteins may correspond to the N-terminal regions of the same or different group B Streptococcus surface proteins. Preferably, in order to obtain a wide scope of protection, each capsular polysaccharide and amino acid sequence, be it in an immunogenic complex or in an immunogenic fusion protein, is derived from different serotypes/strains, and for the amino acid sequences, from different group B Streptococcus surface proteins.

Thus one embodiment of the immunogenic product may comprise an immunogenic complex where the group B Streptococcus surface protein is Alp1 and where, for the immunogenic fusion protein, the first and the second group B Streptococcus surface proteins are Rib and AlpC, respectively.

Another embodiment of the immunogenic product may comprise a first immunogenic complex where the group B Streptococcus surface protein is Alp1 or Alp2, a second immunogenic complex where the group B Streptococcus surface protein is Rib, and optionally a third immunogenic complex where the group B Streptococcus surface protein is AlpC.

In another embodiment of the immunogenic product according to the second aspect of the present invention the group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp4 protein, and optionally the further group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp 4 protein. The group B Streptococcus surface protein and the further group B Streptococcus surface protein may be selected from the group consisting of Alp2 protein, Alp3 protein, and Alp4 protein.

In a preferred embodiment of the immunogenic product according to the second aspect of the present invention there is only one amino acid sequence, having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, in the immunogenic complex. This means that the immunogenic complex, in the immunogenic product, contains only a single amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein in the immunogenic complex. The group B Streptococcus surface protein may be selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein, and AlpC protein.

A third aspect of the present invention concerns a vaccine comprising a pharmaceutically acceptable vehicle, optionally an adjuvant, and a pharmaceutically effective amount of an immunogenic complex according to the first aspect of the present invention or an immunogenic product according to the second aspect of the present invention, wherein the vaccine is capable of eliciting protective immunity against group B Streptococcus.
The term “pharmaceutical acceptable vehicle” is intended to mean any suitable acceptable excipient, adjuvants, carrier, diluent commonly used in pharmaceutical formulations.
The vaccine may be a vaccine composition.
The vaccine may, in addition to the fusion protein, comprise other pharmacologically acceptable ingredients such as salts, buffers, immunoactive components, adjuvants (AlOH), wetting agents, emulsifying and suspending agents, or sweetening, flavouring, perfuming agents, or other substances which are desirable for improving the efficacy of the composition. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient individual.
A multivalent vaccine may also be prepared by combining the immunogenic complex or the immunogenic product with other components, including other fusion proteins as described above, including but not limited to diphtheria toxoid or

tetanus toxoid, or polysaccharides, using techniques known in the art. The vaccine may further comprise further antigens such as RSV antigens or E. coli antigens. Methods for the preparation and formulation of vaccines and vaccine compositions are well known to those skilled in the art. The choice of ingredients will for instance vary depending on the administration route of the composition. For example compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water.

In a further embodiment of the third aspect of the present invention the vaccine may comprise an additional immunoactive component. The additional immunoactive component may be an antigen, an immune enhancing substance, and/or a vaccine; either of these may comprise an adjuvant.

Adjuvants are substances that can be used to specifically augment a specific immune response. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the animal or human being immunized. Adjuvants can be loosely divided into several groups based upon their composition. These groups include oil adjuvants (for example, Freund's complete and incomplete), mineral salts for example, AlK (SO4)2, AlNa (SO4)2, AlNH4 (SO4), AlOH, silica, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU acids), and certain natural substances (for example, wax D from Mycobacterium tuberculosis, as well as substances found in Corynebacterium parvum, or Bordetella pertussis, and members of the genus Brucella. Among those substances particularly useful as adjuvants are saponins such as, for example, Quil A. Examples of materials suitable for use in vaccine compositions are provided in Remington's Pharmaceutical Sciences (Osol, A, Ed, Mack Publishing Co, Easton, PA, pp. 1324-1341 (1980).

The vaccine according to the third aspect of the present invention may be administrated parenterally, intramuscularly, intravenously, intraperitoneally, intradermally, mucosally, submucosally, topically or subcutaneously.

The vaccine according to the third aspect may further comprise more than one immunogenic complex according to the first aspect of the present invention. Thus the vaccine may for example comprise a first immunogenic complex where the group B Streptococcus surface protein is Alp1 or Alp2, a second immunogenic

complex where the group B Streptococcus surface protein is Rib, and optionally a third immunogenic complex where the group B Streptococcus surface protein is AlpC. The capsular polysaccharides of the first, second, and optionally, third immunogenic complexes may be derived from the same serotype/strain, however it is preferred that the capsular polysaccharides are derived from different serotypes/strains.

Further the vaccine may comprise both an immunogenic complex according to the first aspect of the present invention and an immunogenic product according to the second aspect of the present invention.

The vaccine preferably comprises a pharmaceutically effective amount of an immunogenic product in which the Group B Streptococcus surface protein and optionally the further Group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp4 protein, or in which there is only one amino acid sequence having at least 80% sequence identity with the N-terminal of a Group B Streptococcus surface protein. The group B Streptococcus surface protein may be selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein, and AlpC protein.

Preferable the vaccine comprises aluminium hydroxide as an adjuvant.

Thus, in one embodiment, the vaccine consists of a pharmaceutically effective vehicle, aluminium hydroxide, and an immunogenic product in which there is only one amino acid sequence having at east 80% sequence identity with the N-terminal of a Group B Streptococcus surface protein.

The corresponding fourth and fifth aspect of the present invention pertain to the immunogenic complex according to the first aspect of the present invention, the immunogenic product according to the second aspect of the present invention, and/or the vaccine according to the third aspect of the present invention for use in a method of preventing or treating an infection caused by a group B Streptococcus, and
a method of preventing or treating an infection caused by a group B Streptococcus comprising administering to the immunogenic complex according to the first aspect of the present invention, the immunogenic product according to the second aspect of the present invention, and/or the vaccine according to the third aspect of the present invention, respectively.

There is also, according to a further aspect of the present invention, provided a use of the immunogenic complex according to the first aspect of the present invention

and/or the immunogenic product according to the second aspect of the present invention for the manufacture of a vaccine for preventing or treating an infection caused by a group B Streptococcus.

The immunogenic complex according to the first aspect of the present invention, the immunogenic product according to the second aspect of the present invention, and/or the vaccine according to the third aspect of the present invention may be administered in an effective amount to an individual.

The term "effective amount" in relation to the present invention refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additives and diluents; i.e., a carrier, or administration vehicle. Further, it is intended to mean an amount sufficient to reduce and most preferably prevent a clinically significant deficit in the activity and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluents; i.e., carrier, or additive. Further, the dosage to be administered will vary depending on the active principle or principles to be used, the age, weight etc of the individual to be treated.

The terms "preventing or treating" in its various grammatical forms in relation to the present invention refer to preventing, curing, reversing, attenuating, alleviating, ameliorating, inhibiting, minimizing, suppressing, or halting (1) the deleterious effects of a disorder associated with group B Streptococcus infection, (2) disorder progression, or (3) disorder causative agent (group B Streptococcus). Further, the terms "preventing or treating" are contemplated to include the creation of total or partial immunity of the individual to group B Streptococcus infection.

Maternal immunoprophylaxis with a vaccine, for protecting against infection to group B Streptococcus both in the mother and in the young infant, has long been proposed as a potential route.
Thus some embodiments of the corresponding forurth and fifth aspects of the present invention comprise administering to a human female an effective amount of an immunogenic complex, immunogenic product, or vaccine as described herein capable of conferring immunity to the infection to an unborn offspring of the human female.
According to these embodiments, the vaccine is administered to a non-pregnant

female or to a pregnant female, under conditions of time and amount sufficient to cause the production of antibodies which serve to protect both the female and a fetus or newborn (via passive transfer of antibodies across the placenta).

A further aspect of the present invention concerns a method for preventing or treating an infection caused by a group B Streptococcus which comprises administering to an individual in need thereof an effective amount of antibodies elicited from the exposure of a second individual to an immunogenic complex, immunogenic product and/or a vaccine according to the first, second and/or third aspects of the present invention.
According to this aspect, resistance to group B Streptococcus is conferred to the individual by passive immunization, i.e., the immunogenic complex, immunogenic product and/or vaccine is provided to a host (i.e. a human or mammal) volunteer, and the elicited antisera is recovered and directly provided to a recipient suspected of having an infection caused by a group B Streptococcus. It is contemplated that such antisera could be administered to a pregnant female (at or prior to parturition), under conditions of time and amount sufficient so that the antisera would serve to protect either the fetus or newborn (via passive incorporation of the antibodies across the placenta).

The vaccine or antisera of the present invention may, thus, be provided either prior to the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection.

The vaccine may be administered to humans or animals, including mammals and birds, such as rodents (mouse, rat, guinea pig, or rabbit); birds (turkey, hen or chicken); other farm animals (cow, horse, pig or piglet); pets (dog, cat and other pets); and humans. While many animals may be treated with the vaccine of the invention, a preferred individual for treatment is a human or commercially valuable animal and livestock such as fish, e.g. Tilapia, and camels.

The vaccine can be administered to an individual according to methods known in the art. Such methods comprise application e.g. parenterally, such as through all routes of injection into or through the skin: e.g. intramuscular, intravenous, intraperitoneal, intradermal, mucosal, submucosal, or subcutaneous. Also, they may be applied by topical application as a drop, spray, gel or ointment to the mucosal epithelium of the eye, nose, mouth, anus, or vagina, or onto the epidermis of the outer skin at any part of the body. Other possible routes of application are by spray, aerosol, or powder application through inhalation via the respiratory tract. In this last case the particle size that is used will determine how deep the particles will penetrate into the respiratory tract. Alternatively, application can be via the

alimentary route, by combining with the food, feed or drinking water e.g. as a powder, a liquid, or tablet, or by administration directly into the mouth as a: liquid, a gel, a tablet, or a capsule, or to the anus as a suppository. The vaccine may also be administrated in the form of a DNA vaccine.

Many different techniques exist for the timing of the immunizations. It is possible to use the immunogenic complex, immunogenic product and/or vaccine more than once to increase the levels and diversities of expression of the immunoglobulin repertoire expressed by the immunized animal. Typically, if multiple immunizations are given, they will be given one to two months apart.

In the immunogenic product the preferred human dose of the immunogenic fusion protein in the presence of Alhydrogel is within the range of 1 to 250 μg, preferably 10 to 150 μg, preferably 25 to 100 μg or 40 to 80 μg. In the absence of Alhydrogel, the preferred human doses of the immunogenic fusion protein would be 10 to 100 μg, preferably 50 to 500 μg, or preferably 100 to 250 μg.

Generally, the dosage may consist of an initial injection, most probably with adjuvant, followed most probably by one or maybe more booster injections. Preferably, booster injections may be administered at about 1 and 6 months after the initial injection.

CLAIMS

1. An immunogenic complex comprising:

an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, and

a capsular polysaccharide,

wherein the immunogenic complex is capable of eliciting protective immunity against group B Streptococcus.

2. The immunogenic complex according to claim 1, wherein the group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein and AlpC protein.

3. The immunogenic complex according to claim 2, wherein the group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp4 protein.

4. The immunogenic complex according to any of the claims 1-3, further comprising a further amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a further group B Streptococcus surface protein.

5. The immunogenic complex according to claim 4, wherein the further group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp 4 protein and AlpC protein.

6. The immunogenic complex according to claim 5, wherein the further group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp 4 protein.

7. The immunogenic complex according to any of the claims 4-6, wherein the group B Streptococcus surface protein and the further group B Streptococcus surface proteins are different.

8. The immunogenic complex according to any of the proceedings claims, wherein there is only one amino acid sequence, having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein, in the immunogenic complex.

9. The immunogenic complex according to any of the preceding claims, wherein the

group B Streptococcus surface protein and the capsular polysaccharide, and optionally also the further group B Streptococcus surface protein, are derived from different group B Streptococcus serotypes.

10. The immunogenic complex according to any preceding claim, wherein the amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14, and wherein optionally the further amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14.

11. The immunogenic complex according to any preceding claim, wherein the amino acid sequence, and optionally also the further amino acid sequence, is conjugated to the capsular polysaccharide.

12. The immunogenic complex according to any of preceding claims, wherein the amino acid sequence, and optionally also the further amino acid sequence, is modified by glycosylation, amidation, carboxylation or phosphorylation, or by being conjugated to an RSV antigen.

13. An immunogenic product comprising the immunogenic complex according to any of the preceding claims, wherein the immunogenic product further comprises an immunogenic fusion protein comprising:

a first amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a first group B Streptococcus surface protein, which is fused to

a second amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a second group B Streptococcus surface protein

wherein each of the first and the second group B Streptococcus surface protein is selected from the group consisting of Rib protein, Alp1 protein, Alp2 protein, Alp3 protein, Alp4 protein and AlpC protein, and wherein the immunogenic fusion protein is capable of eliciting protective immunity against group B Streptococcus.

14. The immunogenic product according to claim 13, wherein the first amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14, and wherein the second amino acid sequence has at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with one of the amino acid sequences SEQ IDs 2, 4, 8, 10 and 14,

or alternatively,
wherein the immunogenic fusion protein comprises an amino acid sequence having at least 80%, such as at least 85%, such as at least 90%, such as 95, 96, 97, 98 or 99 % sequence identity with any one of the amino acid sequences SEQ ID NO:6 and 12.

15. The immunogenic product according to any of the claims 13-14, wherein the group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp4 protein, and wherein optionally the further group B Streptococcus surface protein is selected from the group consisting of Alp1 protein, Alp2 protein, Alp3 protein, and Alp 4 protein.

16. The immunogenic product according to any of the claims 13-15, wherein there is only one amino acid sequence having at least 80% sequence identity with the amino acid sequence of the N-terminal region of a group B Streptococcus surface protein in the immunogenic complex.

17. A vaccine comprising a pharmaceutically acceptable vehicle, optionally an adjuvant, and a pharmaceutically effective amount of an immunogenic complex according to any one of claims 1-12 or an immunogenic product according to any one of the claims 13-16, wherein the vaccine is capable of eliciting protective immunity against group B Streptococcus.

18. The vaccine according to claim 17, wherein the vaccine comprises a pharmaceutically effective amount of an immunogenic product according to any of claims 15-16.

19. The vaccine according to any of claims 17-18, further comprising aluminium hydroxide as an adjuvant.

20. The vaccine according to any of claims 17-19, wherein the vaccine consists of a pharmaceutically effective vehicle, aluminium hydroxide, and an immunogenic product according to claim 16.

21. The immunogenic complex according to any of the claims 1-12, the immunogenic product according to any of the claim 13-16, and/or the vaccine according to any of the claims 17-20 for use in a method of preventing or treating an infection caused by a group B Streptococcus.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080927

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/09
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/016984 A2 (BRIGHAM & WOMENS HOSPITAL [US]; MADOFF LAWRENCE C [US]; PAOLETTI LAWRE) 7 February 2008 (2008-02-07) Examples claim 1 page 10, paragraph 3 page 11, paragraph 2 - paragraph 4 -----	1-3, 8-12, 17-21
X	EP 0 866 133 A2 (BRIGHAM & WOMENS HOSPITAL [US]) 23 September 1998 (1998-09-23) examples 12-18 Claims ----- -/--	1-3, 8-12, 17-21

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 March 2017	21/03/2017

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/127179 A1 (LUNDS UNI UTVECKLINGSAKTIEBOLA [SE]; LINDAHL GUNNAR [SE]) 23 October 2008 (2008-10-23) cited in the application page 3, line 22 - line 29 page 17, line 15 - line 18 Discloses the polypeptides of SEQ ID NO: 2, 4, and 6. Examples, claims	1-4, 7-12, 17-21
X	STAALHAMMAR-CARLEMALM MARGARETHA ET AL: "Nonimmunodominant regions are effective as building blocks in a streptococcal fusion protein vaccine", CELL HOST & MICROBE, ELSEVIER, NL, vol. 2, no. 6, 13 December 2007 (2007-12-13), pages 427-434, XP002583466, ISSN: 1931-3128, DOI: 10.1016/J.CHOM.2007.10.003 [retrieved on 2007-12-12] page 427, right-hand column, paragraph before last end of the introduction; page 428 page 429, right-hand column, paragraph 1 page 432, left-hand column, paragraph 3	1-21
A	G. LINDAHL ET AL: "Surface Proteins of Streptococcus agalactiae and Related Proteins in Other Bacterial Pathogens", CLINICAL MICROBIOLOGY REVIEWS, vol. 18, no. 1, 1 January 2005 (2005-01-01), pages 102-127, XP055166916, ISSN: 0893-8512, DOI: 10.1128/CMR.18.1.102-127.2005 figure 4	1-21
A	KONG ET AL: "Molecular profiles of group B streptococcal surface protein antigen genes: relationship to molecular serotypes", JOURNAL OF CLINICAL MICROBIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 40, no. 2, 1 February 2002 (2002-02-01), pages 620-626, XP002985191, ISSN: 0095-1137, DOI: 10.1128/JCM.40.2.620-626.2002 The query sequence SEQ ID NO:14 has 100 % identity (100 % similarity) over 103 positions in a common overlap (range (q:s): 1-103:58-160) with subject UNIPARC:UPI00021A18B2 (length: 257).	1-21

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/080927

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