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Use of active P40 conjugates for nasal delivery

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(54) Title: USE OF ACTIVE P40 CONJUGATES FOR NASAL DELIVERY

(54) Titre: UTILISATION DE CONJUGUES P40 ACTIFS PAR VOIE NASALE

(57) Abstract

The invention concerns the use of at least an enterobacteria outer membrane protein A fragment or a Klebsiella membrane protein fragment for preparing a pharmaceutical composition for nasal delivery, to improve a mammal's immunity to an antigen or a haptén.

(57) Abrégé

L'invention concerne l'utilisation d'au moins un fragment d'une protéine de membrane OmpA d'entérobactérie ou de protéine de membrane de Klebsiella pour la préparation d'une composition pharmaceutique destinée à être administrée par voie nasale, pour améliorer l'immunité d'un mammifère vis-à-vis d'un antigène ou d'un haptène.

USE OF ACTIVE P40 CONJUGATES FOR NASAL DELIVERY

The present invention relates to the production of immunizing preparations which are effective in nasal administration. It thus relates to the use of carrier proteins which can improve the immune response to a hapten when the hapten/carrier protein conjugate is administered nasally.

The use of vaccine for oral or nasal delivery is thought to have a great influence on the eradication of pathogenic microorganisms. Specifically, any modification of a vaccine which allows it to be used with greater flexibility (heat-stability, distribution without syringes, etc.), would result in a more effective and more widely used vaccination. On the other hand, immunization via the mucous membrane pathways makes it possible to induce a local immunity which constitutes the first barrier against invasion by a microorganism.

Currently, the oral vaccines which are on the market only concern attenuated or recombined live vectors:

- tetravalent oral vaccine against polio,
- oral vaccine against typhoid fever.

Approaches for nasal or oral vaccination are already described in the literature.

Tests have thus been carried out on mucosal administrations of PspA, which corresponds to the surface protein A of Pneumococcus (Briles D.E., patent EP 0,682,950), on hemagglutinin filaments (Capron A., patent FR 2,718,750; Kimura A., patent EP 0,471,177; Shahin R.D., US patent 7532327), on a fragment of the tetanus toxin (Dougan G., patent WO 93/21950) and on cholera toxin B (CTB).

A protein of the external membrane of *Neisseria meningitidis* is used, mixed with the hapten as an adjuvant for a nasal immunization (Van de Verg L.L., *Infection and immunity*, 1996, 64: 5263-5268).

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Unexpectedly, the Applicant has now found that a membrane protein originating from another bacterium makes it possible, when it is administered nasally together with an antigen, to induce an immune response of satisfactory strength and quality for the production
5 of a vaccine.

For this reason, the subject of the present invention is the use of at least one fragment of an enterobacterium membrane protein OmpA for preparing a
10 pharmaceutical composition adapted for nasal administration

to improve the immunity of a mammal with respect to an antigen or to a hapten.

In the present invention, the term "OmpA" is intended to refer to the type A proteins of the external membrane (OmpA for Outer membrane protein A).
15

A subject of the invention is also the use of at least one fragment of a membrane protein of *Klebsiella* for preparing a pharmaceutical composition intended to be administered nasally, to improve the
20 immunity of a mammal with respect to an antigen or to a hapten.

Preferably, the membrane protein is an OmpA protein of *Klebsiella pneumoniae*.

Advantageously, said fragment of the enterobacterium membrane protein OmpA or of the
25 *Klebsiella* membrane protein according to the invention is obtained by recombinant process.

Very advantageously, said membrane protein or its fragment, obtained by recombinant process, is,
30 after extraction, renatured in the presence of detergent chosen from Zwittergent 3-14, Zwittergent 3-12 and octylglycopyranoside, preferably in the presence of Zwittergent 3-14 at a concentration of between 0.05% and 2% (w/v), very preferably at a
35 concentration close to 0.1%.

Application WO 96/14415 has shown that the major membrane protein of *Klebsiella pneumoniae*, which is the OmpA named P40, coupled to peptide subunit antigens is very immunogenic via the systemic route.

The recombinant P40 protein, expressed in E.Coli in the form of inclusion bodies, is named rP40.

In the context of the present invention, a particularly suitable protein comprises the sequence
5 SEQ ID No 1.

The Applicant has demonstrated that an anti-P40 antibody response is found in all adults, the enterobacterium *Klebsiella pneumoniae* being a very widespread pathogen. This sensitization favors an
10 increase in the antibody response directed against an antigen or a hapten which is administered while coupled to the carrier protein P40. The administration is carried out nasally in the absence of adjuvant.

Said antigen or hapten according to the
15 invention can be chosen from the group comprising proteins, peptides, polysaccharides, oligosaccharides and nucleic acids. Advantageously, it is of bacterial or viral origin.

The present invention is thus suitable for
20 preparing vaccine directed against any microorganism responsible for pathologies of the airways, such as for example microorganisms chosen from RSV, parainfluenzae virus (PIV), influenza virus, hantavirus, streptococci, pneumococci and meningococci.

The antigen or hapten according to the
25 invention will comprise at least one fragment of said microorganism, such as a protein fragment, which persons skilled in the art will know how to determine for its capacity to confer the desired immunity, by
30 standard techniques such as those described in the examples below.

In particular, the present invention is
35 suitable for preparing vaccine directed against RSV (or respiratory syncytial virus), in particular human or bovine RSV. In this case, the antigen or hapten according to the invention comprises at least one protein fragment of the virus RSV, and in particular at least one fragment of the protein G of the RSV.

The sequences of such fragments have in particular been described in application WO 95/27787.

Preferably, said protein fragments of the virus RSV are chosen from the fragments having the sequences
5 SEQ ID No 2 to SEQ ID No 74 as amino acid sequences.

Sequences which are suitable for preparing a vaccine according to the invention are the sequences SEQ ID No 2 to SEQ ID No 74.

The chemical conjugates derived from the
10 coupling of peptides to at least one fragment of a membrane protein of *Klebsiella*, such as rP40, give good results, and an evaluation of the immune response shows very strong antibody responses against these peptides after presensitization with *Klebsiella pneumoniae*.

Advantageously, the protein fragment
15 originating from enterobacteria membrane protein OmpA or from membrane protein of *Klebsiella* is covalently coupled to the antigen or hapten, such as a protein fragment of the RSV.

The invention also comprises the use of at
20 least one fragment of an enterobacteria membrane protein OmpA or of a membrane protein of *Klebsiella* according to the invention, characterized in that said fragment is covalently coupled to said antigen or
25 hapten.

According to the invention, it is possible to
introduce one or more bonding elements, in particular amino acids, to facilitate the coupling reactions
30 between the fragment of membrane protein and the antigen or hapten.

The covalent coupling of the antigen or hapten
according to the invention can be carried out at the N- or C-terminal end of the fragment of the membrane
35 protein according to the invention. The bifunctional reagents which allow this coupling can be determined as a function of the end of the fragment of the membrane protein which is chosen to perform the coupling, and of the nature of the antigen or hapten to be coupled.

These coupling techniques are well known to persons skilled in the art.

The conjugates derived from the coupling of peptides to at least one fragment of an enterobacteria membrane protein OmpA or of a membrane protein of Klebsiella can be prepared by genetic recombination. The hybrid protein (conjugate) can in fact be produced by recombinant DNA techniques, by insertion or addition of a sequence encoding the antigenic or hapten peptide(s) into or to the DNA sequence encoding the fragment of membrane protein. These techniques for preparing hybrid protein by genetic recombination are well known to persons skilled in the art (cf. for example S.C. MAKRIDES, 1996, Microbiologicals Reviews, 60, 3, 512-538) and will not be developed in the present description.

Thus, the invention also comprises the use, according to the invention, characterized in that the hybrid protein, obtained after coupling between the fragment of a membrane protein and the antigen or hapten, protein in nature, is prepared by genetic recombination.

The Applicant has also shown that, in the absence of sensitization to *Klebsiella pneumoniae*, the nasal administration of a hapten coupled to at least one fragment of a membrane protein, such as the rP40 protein, in the absence of adjuvant, induced an anti-hapten antibody response.

The invention relates to the use, according to the invention, characterized in that the pharmaceutical composition contains a fragment of a membrane protein coupled to an antigen or hapten according to the invention, or a transformed host cell which is capable of expressing a hybrid recombinant protein containing a fragment of membrane protein coupled to the antigen or hapten according to the invention, in particular in the absence of adjuvant. Among the transformed host cells which are capable of expressing said hybrid protein, gram-negative bacteria such as *Klebsiella pneumoniae*,

Escherichia coli type K12 currently used in fermentation, or *E. coli* transformed with an expression vector plasmid containing a strong promoter such as the operon of the tryptophan promoter (*trp*) are preferred.

5 Also preferred are gram-positive bacteria such as the nonpathogenic staphylococci, *S. carnosus* and *S. xylosus*, since these bacteria do not produce any LPS (lipopolysaccharides) at the membrane surface. These staphylococci can be transfected with expression

10 vectors containing promoters such as *trp*, or the secretion signal of lipase or even the secretion signal of protein A, or alternatively the signal of the promoter of *OmpA* of *Klebsiella pneumoniae*.

Finally, the invention relates to a method for

15 preparing a protein or one of its fragments by the recombinant pathway, characterized in that the protein or its fragment is, after extraction, renatured in the presence of a solution containing a detergent chosen from Zwittergent 3-14, Zwittergent 3-12 and

20 octylglucopyranoside, and in that said recombinant protein is not interferon β .

Preferably, said protein is an enterobacterium membrane protein, in particular of *OmpA* type. Very preferably, said protein is an *OmpA* of *Klebsiella*

25 *pneumoniae*.

In the method according to the invention, the Zwittergent 3-14 will be preferably at a concentration of between 0.05% and 2%, more preferably close to 0.1%.

The following examples are intended to

30 illustrate the invention without in any way limiting the scope thereof.

In these examples, reference will be made to the following figures:

Figures 1A and 1B: Analysis by SDS-PAGE electrophoresis

35 of the rP40 protein after purification.

Figure 1A: detection with Coomassie blue

- lane 1: batch 1, 2 μ g
- lane 2: batch 1, 10 μ g
- lane 3: batch 2, 2 μ g

- lane 4: batch 2, 10 µg
- lane 5: batch 3, 2 µg
- lane 6: batch 3, 10 µg

5 Figure 1B: immunoblot and detection with the aid of an anti-P40 rabbit polyclonal serum

- std: molecular mass standard
- lane 1: denatured rP40, 100 ng
- lane 2: native rP40, 100 ng.

10 **Figure 2**: Division of the patients according to the O.D. (Optical Density) corresponding to the anti-P40 antibodies, measured by ELISA.

Figure 3: Anti-G1' antibody response.

Figure 4: Anti-rP40 antibody response.

Figure 5: Anti-G1' IgA-type antibody response.

15 **Figure 6**: Isotyping of the anti-G1' immunoglobulins obtained in secondary response.

Figure 7: Isotyping of the anti-G1' immunoglobulins obtained in tertiary response.

20 **Figure 8**: Anti-G1' total IgG-type serum antibody response.

Figure 9: Isotyping of the serum anti-G1' immunoglobulins after three immunizations.

Figure 10: Isotyping of the anti-G1' immunoglobulins from broncho-alveolar washes after three immunizations.

25

Example 1: Cloning of rP40

Cloning of the rP40 gene:

The gene encoding rP40 was obtained by amplification by PCR (Polymerase Chain Reaction) from
30 the chromosomal DNA of the *Klebsiella pneumoniae* IP 1145 strain (described in patent WO 96/14415). After identification by DNA sequencing, the fragment corresponding to rP40 is cloned into diverse expression vectors, in particular the one under the control of the
35 trp operon promoter, upstream of 9 amino acids of the leader peptide (MKAI FVLNA). The peptide sequence of rP40 is represented in the sequence listing by the sequence SEQ ID No 1. In various *E.coli* K12 strains, the rP40 protein is produced in the form of inclusion

bodies with a considerable yield (> 10%, g proteins/g of biosolids).

Fermentation of rP40 fusion proteins:

E. coli K12 transformed with the plasmid
5 pvaLP40 is inoculated in an Erlenmeyer flask containing
250 ml of TSB (Tryptic Soy Broth, Difco) medium
containing ampicillin (100 µg/ml, Sigma) and
tetracycline (8 µg/ml, Sigma). This is incubated for
16 hours at $T^{\circ} = 37^{\circ}\text{C}$ with stirring. 200 ml of this
10 culture are inoculated in a fermenter (CHEMAP CF3000,
ALFA LAVAL) containing 2 liters of culture medium. The
medium contains (g/l): glycerol, 5; ammonium sulfate,
2.6; potassium dihydrogen phosphate, 3; dipotassium
hydrogen phosphate, 2; sodium citrate, 0.5; yeast
15 extract, 1; ampicillin, 0.1; tetracycline 0.008;
thiamine, 0.07; magnesium sulfate, 1 and 1 ml/l of
trace element solution and 0.65 ml/l of vitamin
solution. The parameters which are controlled during
the fermentation are: pH, stirring, temperature, degree
20 of oxygenation, supply of combined sources (glycerol or
glucose). The pH is regulated at 7.0. The temperature
is fixed at 37°C . The growth is controlled by supplying
with glycerol (87%) at a constant flow rate (12 ml/h)
so as to maintain the dissolved oxygen tension signal
25 at 30%. When the turbidity of the culture (measured at
580 nm) reaches the value of 80 (after approximately
24 hours of culture), protein production is induced by
adding indole acrylic acid (IAA) to a final
concentration of 25 mg/l. Approximately 4 hours after
30 induction, the cells are harvested by centrifugation.
The amount of biomass obtained is approximately 200 g,
expressed as wet biomass.

Example 2: Extraction and purification of rP40

Materials and methods

35 Extraction of rP40

After centrifugation of the culture medium
(4000 rpm, 10 min, 4°C), the cells are resuspended in a
25 mM Tris-HCl buffer, pH 8.5. A treatment with

lysozyme (0.5 g/l, 1 hour/room temperature/gentle stirring) allows the release of the inclusion bodies.

The pellet of inclusion bodies obtained by centrifugation (25 min at 10,000 g at 4°C) is taken up
5 in a 25 mM Tris-HCl buffer, pH 8.5, containing 5 mM MgCl₂, and then centrifuged (15 min at 10,000 g).

The denaturation of the protein is obtained by incubating the inclusion bodies at 37°C for 2 hours in a 25 mM Tris-HCl buffer, pH 8.5, containing 7 M urea
10 (denaturing agent) and 10 mM dithiothreitol (reduction of disulfide bridges). A centrifugation (15 min at 10,000 g) makes it possible to remove the insoluble portion of the inclusion bodies.

After dilution with 13 volumes of a 25 mM Tris-
15 HCl buffer, pH 8.5, containing NaCl (8.76 g/l) and Zwittergent 3-14 (0.1%, w/v), the mixture is left to stand overnight at room temperature with stirring, in contact with the air (renaturation of the protein by dilution and reoxidation of the disulfide bridges).

20 Purification of the rP40 protein

Anion exchange chromatography step.

After another centrifugation, the sample is dialyzed against a 25 mM Tris-HCl buffer, pH 8.5, containing 0.1% Zwittergent 3-14 (100 volumes of
25 buffer) overnight at 4°C.

The dialyzate is loaded onto a column containing a support of strong anion exchanger type (Biorad Macro Prep High Q gel), which is equilibrated in the buffer described above at a linear flow rate of
30 15 cm/h. The proteins are detected at 280 nm. The rP40 protein is eluted, with a linear flow rate of 60 cm/h for an NaCl concentration of 0.6 M, in the 25 mM Tris/HCl buffer, pH 8.5; 0.1% Zwittergent 3-14.

Cation exchange chromatography step.

35 The fractions containing the rP40 protein are pooled and concentrated by ultrafiltration with the aid of an Amicon cell system with stirring used with a Diaflo membrane of type YM10 (cutoff threshold 10 kDa) for volumes of about 100 ml, or with the aid of a

Millipore Minitan tangential flow filtration system used with membrane plates having a cutoff threshold of 10 kDa, for larger volumes. The fraction thus concentrated is dialyzed overnight at 4°C against a
5 20 mM citrate buffer, pH 3.0, containing 0.1% of Zwittergent 3-14.

The dialysate is loaded onto a column containing a support of strong cation exchanger type (Biorad Macro Prep High S gel), which is equilibrated
10 in the 20 mM citrate buffer, pH 3.0, containing 0.1% of Zwittergent 3-14. The rP40 protein is eluted (rate 6l cm/h) for a 0.7 M NaCl concentration. The fractions containing the rP40 are pooled and concentrated as described above.

15 **Results**

Starting from a 1 liter culture, one denaturation/renaturation cycle makes it possible to obtain 300 mg of protein (estimation by assay according to the Lowry method). 75 mg of rP40 are purified after
20 the two chromatographic steps.

As above, the rP40 protein is concentrated after purification in order to attain a final concentration of between 5 and 10 mg/ml. The electrophoretic profiles show a degree of purity of
25 about 95% (Figure 1A). After immunoblot, the protein is specifically recognized by an anti-natural P40 monoclonal antibody obtained in mice (Figure 1B).

The condition of the protein is monitored by SDS-PAGE. Depending on its form, denatured or native,
30 the P40 protein extracted from the membrane of *Klebsiella pneumoniae* has a characteristic electrophoretic behavior (migration). The native form (β -sheet structure) in fact has a lower molecular mass than the denatured form (α -helix structure) under the
35 action of a denaturing agent, such as urea or guanidine hydrochloride, or with heating to 100°C in the presence of SDS (Figure 1B). The rP40 protein is not correctly renatured at the end of renaturation, regardless of whether this is carried out in the presence or absence

of 0.1% (w/v) Zwittergent 3-14. Conversely, total renaturation is obtained after dialysis against a 25 mM Tris/HCl buffer, pH 8.5, containing 0.1% (w/v) Zwittergent 3-14. However, it should be noted that this
5 renaturation is only obtained when the dilution step and the treatment at room temperature are themselves carried out in the presence of Zwittergent 3-14 (negative results in the absence of detergent).

Example 3: Coupling of the G1' peptide to rP40

10 **Materials and methods**

The G1' peptide is a synthetic peptide of 15 amino acids, the sequence of which is as follows (SEQ ID No 74):

N-₁SIDSNNPTOWAISKC₁₅-C

15 Without the Cys (cysteine) residue added in the C-terminal position, this peptide (portion 1-14) corresponds to portion 174-187 of the protein G of the respiratory syncytial virus, and has, with respect to the native peptide, two major modifications which are:

- 20 - the replacement of the Cys residue at position 13 with a Ser (serine) residue,
- the replacement at positions 3 and 9 of the Cys residues, which form a disulfide bridge, with, respectively, Asp (aspartic acid) and Orn (ornithine)
25 residues which form a lactam-type bridge.

These modifications are introduced for the purpose of removing the Cys residues of the native peptide in order to be able to carry out a one-to-one coupling of the latter to the protein via the Cys
30 residue introduced in the C-terminal position, while at the same time maintaining the structure of the peptide with the aid of the introduction of a lactam bridge.

The coupling of the peptide to the protein is carried out using the BHA or bromo-N-hydroxysuccinimide acetate reagent (Svenson et al., 1990, Proc. Natl.
35 Acad. Sci. USA 87, 1347, Bernatowicz and Matsueda, 1986, Anal. Biochem. 155, 95). This heterobifunctional reagent allows activation of the Lys (lysine) residues of the protein by bromoacetylation, and then coupling

of the peptide via the free thiol group carried by the Cys residue.

5 Firstly, the rP40 protein is activated with the BHA. The rP40 is dialyzed against a 0.1 M phosphate buffer, pH 7, containing 0.1% Zwittergent 3-14, for 24 hours at +4°C. After dialysis, the concentration is adjusted to 5 mg/ml with the aid of the same buffer, before adding BHA in a proportion of 1.2 mg (50 µl)/mg of rP40.

10 The whole is placed in the dark for one hour with stirring and at room temperature.

The activated rP40 is then desalified by gel filtration chromatography (elution with the abovementioned buffer). The fractions containing the 15 bromoacetylated protein are pooled.

For the coupling, the peptide (10 mg/ml in 0.1 M phosphate buffer, pH 7, containing 0.1% Zwittergent 3-14) is added to the activated protein in a proportion of 0.4 mg/mg of protein. After saturation 20 under a nitrogen stream, the tube is again placed in the dark for 2 hours with stirring and at room temperature.

The unbound peptide can be removed with the aid of a dialysis step or of molecular sieve 25 chromatography.

Results

The conjugate obtained is characterized by protein assay (BCA or LOWRY method) and by SDS-PAGE electrophoresis. The degree of coupling of the peptide 30 to the protein is estimated by carboxymethylcysteine residue assay: the assaying of the amino acids released by hydrolysis (6N HCl) is performed by HPLC after derivatization with the aid of PITC (Pico-Tag method, Waters).

35 The degree of coupling determined by this method is approximately 10 G1' peptides/mole of rP40.

Example 4: Natural immunity in adults

Human sera derived from a clinical study are analyzed by ELISA assay in order to determine the presence of anti-P40 antibodies.

5 The results are represented in Figure 2.

Among 113 sera tested after 400-fold dilution, 110 sera give a colorimetric signal revealing the anti-P40 IgGs. There are circulating anti-P40 antibodies in all the patients, with levels which are more or less
10 high depending on the patient under consideration.

Example 5: Anti-G1' antibody response after sensitizations and frequent immunizations

BALB/c mice were or were not sensitized twice with a *Klebsiella pneumoniae* I145 strain, in order to
15 reproduce the seropositivity found in humans. The mice are subsequently immunized nasally in the absence of adjuvant 7 days after the sensitization. This immunization is carried out with a small amount of antigen, the mice receiving 10 µg of G1' equivalent
20 coupled to rP40. The mice receive a booster 10 and 20 days after the first immunization. A retro-orbital sinus puncture is performed on the mice 9 days after the first immunization and 10 days after each booster (secondary and tertiary responses). The serum anti-G1'
25 (Figure 3) and anti-carrier (Figure 4) antibodies are assayed by the ELISA method.

5.1 Assaying of anti-G1' serum IgGs

The results are represented in Figure 3.

In the primary response, the mice presensitized
30 with *Klebsiella pneumoniae* and immunized with rP40-G1' are the only ones to produce anti-G1' antibodies.

The level of anti-G1' antibodies found in the mice presensitized with *Klebsiella pneumoniae* and immunized with rP40-G1' is increased after a second
35 immunization. In the absence of presensitization, a second immunization in the presence of the rP40-G1' conjugates induces an anti-G1' antibody response.

After three immunizations, the anti-G1' antibody response is increased in the presensitized and non-presensitized mice.

5.2 Assaying of anti-rP40 serum IgGs

5 The results are represented in Figure 4.

The anti-P40 antibody response shows that the mice were sensitized to *Klebsiella pneumoniae* in identical manner whatever the batch under consideration.

10 The immunization in the presence of rP40-G1' conjugates slightly increases the anti-rP40 antibody response.

5.3 Assaying of anti-G1' serum IgAs

15 Secondly, we assayed the serum IgA-type anti-G1' antibody response: immunoglobulin characteristic of immunizations carried out via the mucous membrane (nasal or oral) pathways.

The results are represented in Figure 5.

20 After a single immunization, IgAs are not detected. After two immunizations, anti-G1' IgAs are detected essentially in mice presensitized to *Klebsiella pneumoniae* and immunized with rP40-G1'. This response is increased by the third immunization. In the absence of sensitization, anti-G1' IgAs are detected in
25 mice after two immunizations with rP40-G1' conjugates. This level of IgA is increased by the third immunization.

5.4 Isotyping of anti-G1' serum immunoglobulins

30 Two types of response can be observed, Th1 and Th2. These responses differ by the profile of cytokines produced and by their functions in the immune response. IgG1s are characteristic of a response of type Th2, and IgG2as are characteristic of a Th1 response.

35 A mixed Th1 and Th2 response profile is found only in the mice immunized with the rP40-G1' conjugates, whether or not they are presensitized with *Klebsiella pneumoniae* (Figure 6).

After three immunizations (Figure 7), the profile remains mixed in the mice immunized with the rP40-G1' conjugates.

Example 6: Anti-G1' antibody response after sensitizations and infrequent immunizations.

With respect to the above protocol, the first immunization is separated from the final sensitization by a period of 3 weeks instead of one week. The anti-G1' antibodies are assayed in the sera, and, in the tertiary response, in broncho-alveolar washes, by the ELISA method.

6.1 Assaying of anti-G1' serum IgGs

As seen in Figure 8, 7 days after the first immunization, anti-G1' serum antibodies of type total IgG are detected in the mice presensitized to *Klebsiella pneumoniae* and immunized in the presence of the rP40-G1' conjugates. This antibody response is increased by the two other immunizations.

6.2 Isotyping of serum immunoglobulins

The results are represented in Figure 9.

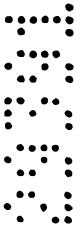
In this case, we also observe a mixed response, we obtain in fact the same titer for IgG1 as for IgG2a (Figure 9). In addition, a high level of IgA is found in the mice presensitized to *Klebsiella pneumoniae* and immunized three weeks later in the presence of the rP40-G1' conjugates.

6.3 Isotyping of immunoglobulins from broncho-alveolar washes

In the broncho-alveolar washes, the 4 types of immunoglobulin are found only in the mice sensitized to *Klebsiella pneumoniae* and immunized 3 times in the presence of the rP40-G1' conjugates (Figure 10).

It is to be understood that a reference herein to a prior art document does not constitute an admission that the document forms part of the common general knowledge in the art in Australia or in any other country.

5 In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprising" and grammatical variations thereof, is used in the sense of "including" i.e. the features specified may be associated with further features in various embodiments of the invention.



EDITORIAL NOTE

APPLICATION NUMBER - 29391/99

The following Sequence Listing pages 1 to 27 are part of the description. The claims pages follow on pages "16" to "18".

SEQUENCE LISTING

Information for SEQ ID NO: 1 rP40

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 344 amino acids, 1032 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

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      1
      N - Met Lys Ala Ile Phe Val Leu Asn Ala Ala Pro Lys
      5'- ATG AAA GCA ATT TTC GTA CIG AAT GCG GCT CCG AAA
      30
Asp Asn Thr Trp Tyr Ala Gly Gly Lys Leu Gly Trp Ser Gln Tyr His Asp Thr
GAT AAC ACC TGG TAT GCA GGT GGT AAA CTG GGT TGG TCC CAG TAT CAC GAC ACC
      48
Gly Phe Tyr Gly Asn Gly Phe Gln Asn Asn Asn Gly Pro Thr Arg Asn Asp Gln
GGT TTC TAC GGT AAC GGT TTC CAG AAC AAC AAC GGT CCG ACC CGT AAC GAT CAG
      66
Leu Gly Ala Gly Ala Phe Gly Gly Tyr Gln Val Asn Pro Tyr Leu Gly Phe Glu
CTT GGT GCT GGT GCG TTC GGT GGT TAC CAG GTT AAC CCG TAC CTC GGT TTC GAA
      84
Met Gly Tyr Asp Trp Leu Gly Arg Met Ala Tyr Lys Gly Ser Val Asp Asn Gly
ATG GGT TAT GAC TGG CTG GGC CGT ATG GCA TAT AAA GGC AGC GTT GAC AAC GGT
      102
Ala Phe Lys Ala Gln Gly Val Gln Leu Thr Ala Lys Leu Gly Tyr Pro Ile Thr
GCT TTC AAA GCT CAG GGC GTT CAG CTG ACC GCT AAA CTG GGT TAC CCG ATC ACT
      120
Asp Asp Leu Asp Ile Tyr Thr Arg Leu Gly Gly Met Val Trp Arg Ala Asp Ser
GAC GAT CTG GAC ATC TAC ACC CGT CTG GGC GGC ATG GTT TGG CCG GCT GAC TCC
      138
Lys Gly Asn Tyr Ala Ser Thr Gly Val Ser Arg Ser Glu His Asp Thr Gly Val
AAA GGC AAC TAC GCT TCT ACC GGC GTT TCC CGT AGC GAA CAC GAC ACT GGC GTT
      156
Ser Pro Val Phe Ala Gly Gly Val Glu Trp Ala Val Thr Arg Asp Ile Ala Thr
TCC CCA GTA TTT GCT GGC GGC GTA GAG TGG GCT GTT ACT CGT GAC ATC GCT ACC
      174
Arg Leu Glu Tyr Gln Trp Val Asn Asn Ile Gly Asp Ala Gly Thr Val Gly Thr
CGT CTG GAA TAC CAG TGG GTT AAC AAC ATC GGC GAC CCG GGC ACT GTG GGT ACC
      192
Arg Pro Asp Asn Gly Met Leu Ser Leu Gly Val Ser Tyr Arg Phe Gly Gln Glu
CGT CCT GAT AAC GGC ATG CTG AGC CTG GGC GTT TCC TAC CCG TTC GGT CAG GAA
      210
Asp Ala Ala Pro Val Val Ala Pro Ala Pro Ala Pro Ala Pro Glu Val Ala Thr
GAT GCT GCA CCG GTT GTT GCT CCG GCT CCG GCT CCG GCT CCG GAA GTG GCT ACC
      228
Lys His Phe Thr Leu Lys Ser Asp Val Leu Phe Asn Phe Asn Lys Ala Thr Leu
AAG CAC TTC ACC CTG AAG TCT GAC GTT CTG TTC AAC TTC AAC AAA GCT ACC CTG
      246
Lys Pro Glu Gly Gln Gln Ala Leu Asp Gln Leu Tyr Thr Gln Leu Ser Asn Met
AAA CCG GAA GGT CAG CAG GCT CTG GAT CAG CTG TAC ACT CAG CTG AGC AAC ATG
      264
Asp Pro Lys Asp Gly Ser Ala Val Val Leu Gly Tyr Thr Asp Arg Ile Gly Ser
GAT CCG AAA GAC GGT TCC GCT GTT GTT CTG GGC TAC ACC GAC CCG ATC GGT TCC

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282
 Glu Ala Tyr Asn Gln Gln Leu Ser Glu Lys Arg Ala Gln Ser Val Val Asp Tyr
 GAA GCT TAC AAC CAG CAG CTG TCT GAG AAA CGT GCT CAG TCC GTC GTT GAC TAC
 300
 Leu Val Ala Lys Gly Ile Pro Ala Gly Lys Ile Ser Ala Arg Gly Met Gly Glu
 CTG GTT GCT AAA GGC ATC CCG GCT GGC AAA ATC TCC GCT CCG GGC ATG GGT GAA
 318
 Ser Asn Pro Val Thr Gly Asn Thr Cys Asp Asn Val Lys Ala Arg Ala Ala Leu
 TCC AAC CCG GTT ACT GGC AAC ACC TGT GAC AAC GTG AAA GCT CCG GCT GCC CTG
 336
 Ile Asp Cys Leu Ala Pro Asp Arg Arg Val Glu Ile Glu Val Lys Gly Tyr Lys
 ATC GAT TGC CTG GCT CCG GAT CGT CGT GTA GAG ATC GAA GTT AAA GCC TAC AAA
 344
 Glu Val Val Thr Gln Pro Gln Ala
 GAA GTT GTA ACT CAG CCT CAG GCT

Information for SEQ ID NO: 2 G2A

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 101 amino acids, 303 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

130

N - Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys
 5'- ACC GTG AAA ACC AAA AAC ACC ACG ACC ACC CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA
 150
 Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe
 CAG CGT CAG AAC AAA CCG CCG AAC AAA CCG AAC APC GAT TTC CAT TTC GAA GTG TTC AAC TTC
 171 173 176 182 186
 Val Pro Cys Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn
 GTG CCG TGC AGC ATC TGC AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC
 192
 Lys Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr Thr Lys
 AAA AAA CCG GGC AAA AAA ACC ACG ACC AAA CCG ACC AAA AAA CCG ACC TTC AAA ACC ACC AAA
 213 230
 Lys Asp His Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro - C
 AAA GAT CAT AAA CCG CAG ACC ACC AAA CCG AAA GAA GTG CCG ACC ACC AAA CCG - 3'

Information for SEQ ID NO: 3 G2B

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 101 amino acids, 303 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

130

N - Thr Ala Gln Thr Lys Gly Arg Ile Thr Thr Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys
 5'- ACC GCG CAG ACC AAA GGC CGT ATC ACC ACC ACC CAG ACC APC AAA CCG ACC ACC AAA
 150

Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe
 ACC CGT ACC AAA AAC CCG CCG AAA AAA CCG AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC

171 173 176 182 186
 Val Pro Cys Ser Ile Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser
 GTG CCG TGC ACC ATC TGC GGC AAC AAC CAG CTG TGC AAA ACC ATC TGC AAA ACC ATC CCG ACC
 192

Asn Lys Pro Lys Lys Lys Pro Thr Ile Lys Pro Thr Asn Lys Pro Thr Thr Lys Thr Thr Asn
 AAC AAA CCG AAA AAG AAA CCG ACC ATC AAA CCG ACC AAC AAA ACC AAA ACC AAC AAC
 213

Lys Arg Asp Pro Lys Thr Pro Ala Lys Met Pro Lys Lys Glu Ile Ile Thr Asn - C
 AAA CGT GAT CCG AAA ACC CCG GCG AAA ATG CCG AAG AAG GAA ATC ATC ACC AAC - 3'

Information for SEQ ID NO: 4 G2AδCys

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 101 amino acids, 303 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

130

N - Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys
 5'- ACC GTG AAA ACC AAA AAC ACC ACG ACC ACC CAG ACC CAG CCG ACC AAA CCG ACC ACC AAA
 150

Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe
 CAG CGT CAG AAC AAA CCG CCG AAC AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC
 171 173 176 182 186

Val Pro Ser Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn
 GTG CCG ACC ACC ATC TGC ACC AAC AAC CCG ACC TCC TGG GCG ATC ACC AAA CGT ATC CCG AAC
 192

Lys Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr Thr Lys
 AAA AAA CCG GGC AAA AAA ACC ACG ACC AAA CCG ACC AAA AAA CCG ACC TTC AAA ACC ACC AAA
 213

Lys Asp His Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro - C
 AAA GAT CAT AAA CCG CAG ACC ACC AAA CCG AAA GAA GTG CCG ACC ACC AAA CCG - 3'

Information for SEQ ID NO: 5 G2BδCys

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 101 amino acids, 303 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

130

N - Thr Ala Gln Thr Lys Gly Arg Ile Thr Thr Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys
 5'- ACC GCG CAG ACC AAA GGC CGT ATC ACC ACC ACC ACC CAG ACC ACC AAA CCG AGC ACC AAA

150

Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe
 AGC CGT AGC AAA APC CCG CCG AAA AAA CCG AAA GAT GAT TAC CAC TTC GAA GTG TTC APC TTC
 171 173 176 182 186

Val Pro Ser Ser Ile Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser
 GTG CCG AGC AGC ATC TGC GGC APC APC CAG CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC

192

Asn Lys Pro Lys Lys Lys Pro Thr Ile Lys Pro Thr Asn Lys Pro Thr Thr Lys Thr Thr Asn
 AAC AAA CCG AAA AAG AAA CCG ACC ATC AAA CCG ACC APC AAA CCG ACC ACC AAA ACC ACC AAC
 213 230

Lys Arg Asp Pro Lys Thr Pro Ala Lys Met Pro Lys Lys Glu Ile Ile Thr Asn - C
 AAA CGT GAT CCG AAA ACC CCG GCG AAA ATG CCG AAG AAG GAA ATC ATC ACC AYC - 3'

Information for SEQ ID NO: 6 G1ACys

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 14 amino acids, 42 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys - C
 5'- AGC ATC TGC AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA - 3'

Information for SEQ ID NO: 7 G1BCys

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 14 amino acids, 42 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Ile Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys - C
 5'- AGC ATC TGC GGC AAC AAC CAG CTG TGC AAA AGC ATC TGC AAA - 3'

Information for SEQ ID NO: 8 G1A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 14 amino acids, 42 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Ile Asp Ser Asn Asn Pro Thr Orn Trp Ala Ile Ser Lys - C

Information for SEQ ID NO: 13 G1'B8C

SEQUENCE TYPE: amino acids
 SEQUENCE LENGTH: 14 amino acids
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Ile Asp Gly Asn Asn Gln Leu Orn Lys Ser Ile Ser Lys - C

Information for SEQ ID NO: 14 G2A8CF

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 101 amino acids, 303 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

130

N - Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys
 5' - ACC GTG AAA ACC AAA AAC ACC ACG ACC ACC CAG ACC CAG CCG ACC AAA CCG ACC ACC AAA

150 163 165 168 170
 Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn Asp Ser His Ser Glu Val Ser Asn Ser
 CAG CGT CAG AAC AAA CCG CCG AAC AAA CCG AAC AAC GAT TCC CAT TCC GAA GTG TCC AYC TCC
 171 173 176 182 186
 Val Pro Ser Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn
 GTG CCG AGC AGC ATC TGC AGC AYC AAC CCG ACC TGC TGG GCG ATC ACC AYA CGT ATC CCG AYC
 192

Lys Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr Thr Lys
 AAA AAA CCG GGC AAA AAA ACC ACG ACC AAA CCG ACC AAA AAA CCG ACC TTC AAA ACC ACC AAA
 213 230

Lys Asp His Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro - C
 AAA GAT CAT AAA CCG CAG ACC ACC AAA CCG AAA GAA GTG CCG ACC ACC AAA CCG - 3'

Information for SEQ ID NO: 15 G4A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 17 amino acids, 51 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Cys Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys - C
 5' - GTG CCG TGC AGC ATC TGC AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA - 3'

Information for SEQ ID NO: 16 G4AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 17 amino acids, 51 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Ser Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys - C
 5' - GTG CCG AGC AGC ATC TGC AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA - 3'

Information for SEQ ID NO: 17 G4B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 17 amino acids, 51 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Cys Ser Ile Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys - C
 5' -GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG CTG TGC AAA AGC ATC TGC AAA - 3'

Information for SEQ ID NO: 18 G4BδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 17 amino acids, 51 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Ser Ser Ile Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Ser Lys - C
 5' -GTG CCC AGC AGC ATC TGC GGC AAC AAC CAG CTG TGC AAA AGC ATC AGC AAA - 3'

Information for SEQ ID NO: 19 G4'A

SEQUENCE TYPE: amino acids
 SEQUENCE LENGTH: 17 amino acids
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Asp Ser Ile Asp Ser Asn Asn Pro Thr Orn Trp Ala Ile Orn Lys - C

Information for SEQ ID NO: 20 G4'AδC

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 17 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
N - Val Pro Ser Ser Ile Asp Ser Asn Asn Pro Thr Orn Trp Ala Ile Ser Lys - C

Information for SEQ ID NO: 21 G4'B

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 17 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
N - Val Pro Asp Ser Ile Asp Gly Asn Asn Gln Leu Orn Lys Ser Ile Orn Lys - C

Information for SEQ ID NO: 22 G4'BδC

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 17 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
N - Val Pro Ser Ser Ile Asp Gly Asn Asn Gln Leu Orn Lys Ser Ile Ser Lys - C

Information for SEQ ID NO: 23 G200A

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 61 amino acids, 183 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5' - CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC
 177 182 186
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly
 AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC AAA AAA CCG GGC
 196 200
 Lys Lys Thr Thr Thr - C
 AAA AAA ACC ACC ACC - 3'

Information for SEQ ID NO: 24 G198A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 59 amino acids, 177 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5' - CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC
 177 182 186
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly
 AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC AAA AAA CCG GGC
 196 198
 Lys Lys Thr - C
 AAA AAA ACC - 3'

Information for SEQ ID NO: 25 G196A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5' - CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC
 177 182 186
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly
 AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC AAA AAA CCG GGC
 196
 Lys - C
 AAA - 3'

Information for SEQ ID NO: 26 G194A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 55 amino acids, 165 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC
 177 182 186 194
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro - C
 AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC AAA AAA CCG - 3'

Information for SEQ ID NO: 27 G192A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 52 amino acids, 159 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC
 177 182 186 192
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys - C
 AGC AAC AAC CCG ACC TGC TGG GCG ATC TGC AAA CGT ATC CCG AAC AAA - 3'

Information for SEQ ID NO: 28 G6A

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 51 amino acids, 153 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG TGC AGC ATC TGC

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GIG TTC AAC TTC GIG CCG AGC AGC ATC TGC
 177 182 186
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn Lys Lys Pro Gly
 AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG AAC AAA AAA CCG GCG
 196 198
 Lys Lys Thr - C
 AAA AAA ACC - 3'

Information for SEQ ID NO: 32 G196AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GIG TTC AAC TTC GIG CCG AGC AGC ATC TGC
 177 182 186
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn Lys Lys Pro Gly
 AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG AAC AAA AAA CCG GCG
 196
 Lys - C
 AAA - 3'

Information for SEQ ID NO: 33 G194AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 55 amino acids, 165 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
 5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
 158 173 176
 Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys
 AAA CCG AAC AAC GAT TTC CAT TTC GAA GIG TTC AAC TTC GIG CCG AGC AGC ATC TGC
 177 182 186 194
 Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn Lys Lys Pro - C
 AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG AAC AAA AAA CCG - 3'

Information for SEQ ID NO: 34 G192AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 53 amino acids, 159 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

```

      140
N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
158                                     173                                     176
Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys
AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG AGC AGC ATC TGC
177                                     182                                     186                                     192
Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro Asn Lys - C
AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG AAC AAA - 3'

```

Information for SEQ ID NO: 35 G6AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 51 amino acids, 153 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

```

      140
N - Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn
5'- CAG ACC CAG CCG AGC AAA CCG ACC ACC AAA CAG CGT CAG AAC AAA CCG CCG AAC
158                                     173                                     176
Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys
AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG AGC AGC ATC TGC
177                                     182                                     186                                     190
Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro - C
AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG - 3'

```

Information for SEQ ID NO: 36 G7AδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 33 amino acids, 99 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

```

      158                                     173
N - Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile
5'- AAA CCG AAC AAC GAT TTC CAT TTC GAA GTG TTC AAC TTC GTG CCG AGC AGC ATC
176                                     182                                     186                                     190
Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Ser Lys Arg Ile Pro - C
TGC AGC AAC AAC CCG ACC TGC TGG GCG ATC AGC AAA CGT ATC CCG - 3'

```

Information for SEQ ID NO: 37 G200B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 61 amino acids, 183 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG

160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG

182 186 200
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys Lys Pro Thr Ile- C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG AAA CCG ACC ATC- 3'

Information for SEQ ID NO: 38 G198B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 59 amino acids, 177 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG

160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG

182 186 198
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys Lys Pro - C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG AAA CCG - 3'

Information for SEQ ID NO: 39 G196B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG
 182 186 196
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys - C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG - 3'

Information for SEQ ID NO: 40 G194B

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 55 amino acids, 165 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG
 182 186 191
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn Lys Pro - C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG AGC AAC AAA CCG - 3'

Information for SEQ ID NO: 41 G192B

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 53 amino acids, 159 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG
 182 186 192
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn - C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG AGC AAC - 3'

Information for SEQ ID NO: 42 G6B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 51 amino acids, 153 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC AAC AAC CAG
 182 186 190
 Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro - C
 CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG - 3'

Information for SEQ ID NO: 43 G7B

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 33 amino acids, 99 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

158 173 176
 N - Lys Pro Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly
 5'- AAA CCG AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC TGC AGC ATC TGC GGC
 182 186 190
 Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro - C
 AAC AAC CAG CTG TGC AAA AGC ATC TGC AAA ACC ATC CCG - 3'

Information for SEQ ID NO: 44 G200BdC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 61 amino acids, 183 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GGC AAC AAC CAG
 182 186 200
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys Lys Pro Thr Ile - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG AAA CCG ACC ATC - 3'

Information for SEQ ID NO: 45 G198B8C

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 59 amino acids, 177 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GCC AAC AAC CAG
 182 186 198
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys Lys Pro - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG AAA CCG - 3'

Information for SEQ ID NO: 46 G196B8C

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GCC AAC AAC CAG
 182 186 196
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser Asn Lys Pro Lys Lys - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC AAC AAA CCG AAA AAG - 3'

Information for SEQ ID NO: 47 G194B8C

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 55 amino acids, 165 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GGC AAC AAC CAG
 182 186 194
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser Asn Lys Pro - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC AAC AAA CCG - 3'

Information for SEQ ID NO: 48 G192B8C

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 53 amino acids, 159 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GGC AAC AAC CAG
 182 186 192
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro Ser Asn - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG AGC AAC - 3'

Information for SEQ ID NO: 49 G6B8C

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 51 amino acids, 153 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Ser Thr Gln Thr Asn Lys Pro Ser Thr Lys Ser Arg Ser Lys Asn Pro Pro Lys Lys Pro
 5'- AGC ACC CAG ACC AAC AAA CCG AGC ACC AAA AGC CGT AGC AAA AAC CCG CCG AAA AAA CCG
 160 173 176
 Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly Asn Asn Gln
 AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TGC GGC AAC AAC CAG
 182 186 190
 Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro - C
 CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG - 3'

Information for SEQ ID NO: 50 G7B8C

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 33 amino acids, 99 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

158 173 176
 N - Lys Pro Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Ser Ser Ile Cys Gly
 5'- AAA CCG AAA GAT GAT TAC CAC TTC GAA GTG TTC AAC TTC GTG CCC AGC AGC ATC TCC GCC
 182 186 190
 Asn Asn Gln Leu Cys Lys Ser Ile Ser Lys Thr Ile Pro - C
 AAC AAC CAG CTG TGC AAA AGC ATC AGC AAA ACC ATC CCG - 3'

Information for SEQ ID NO: 51 G2V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 101 amino acids, 303 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

130
 N - Gln Asn Arg Lys Ile Lys Gly Gln Ser Thr Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn
 5'- CAA AAC AGA AAA ATC AAA GGT CAA TCA ACA CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT
 150
 Pro Ser Gly Ser Ile Pro Pro Glu Asn His Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr
 CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT
 171 173 176 182 186
 Val Pro Cys Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu
 GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA
 192
 Arg Ala Pro Ser Arg Ala Pro Thr Ile Thr Leu Lys Lys Thr Pro Lys Pro Lys Thr Thr Lys
 AGA GCA CCA ACC AGA GCA CCA ACA ATC ACC CTC AAA AAG ACA CCA AAA CCA AAA ACC ACA AAA
 213 230
 Lys Pro Thr Lys Thr Thr Ile His His Arg Thr Ser Pro Glu Thr Lys Leu Gln - C
 AAG CCA ACC AAG ACA ACA ATC CAT CAC AGA ACC ACC CCA GAA ACC AAA CTG CAA - 3'

Information for SEQ ID NO: 52 G2V8C

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 101 amino acids, 303 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

130
 N - Gln Asn Arg Lys Ile Lys Gly Gln Ser Thr Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn
 5'- CAA AAC AGA AAA ATC AAA GGT CAA TCA ACA CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT

150
 Pro Ser Gly Ser Ile Pro Pro Glu Asn His Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr
 CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT
 171 173 176 182 186
 Val Pro Ser Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu
 GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA
 192
 Arg Ala Pro Ser Arg Ala Pro Thr Ile Thr Leu Lys Lys Thr Pro Lys Pro Lys Thr Thr Lys
 AGA GCA CCA AGC AGA GCA CCA ACA ATC ACC CTC AAA AAG ACA CCA AAA CCA AAA ACC ACA AAA
 213 230
 Lys Pro Thr Lys Thr Thr Ile His His Arg Thr Ser Pro Glu Thr Lys Leu Gln - C
 AAG CCA ACC AAG ACA ACA ATC CAT CAC AGA ACC AGC CCA GAA ACC AAA CTG CAA - 3'

Information for SEQ ID NO: 53 G200V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 61 amino acids, 183 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 200
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu Arg Ala Pro Ser Arg Ala Pro Thr Ile - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA GCA CCA ACA ATC - 3'

Information for SEQ ID NO: 54 G198V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 59 amino acids, 177 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 198
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu Arg Ala Pro Ser Arg Ala Pro - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA GCA CCA - 3'

Information for SEQ ID NO: 55 G196V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 196
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu Arg Ala Pro Ser Arg - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA - 3'

Information for SEQ ID NO: 56 G194V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 55 amino acids, 165 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 194
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu Arg Ala Pro - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA AGA GCA CCA - 3'

Information for SEQ ID NO: 57 G192V

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 53 amino acids, 159 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140

N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GGC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 192
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr Glu Arg - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG GAA ACA - 3'

Information for SEQ ID NO: 58 G6V

SEQUENCE LENGTH: 51 amino acids, 153 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GGC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT
 182 186 190
 Ala Cys Leu Ser Leu Cys His Ile Glu Thr - C
 GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG - 3'

Information for SEQ ID NO: 59 G7V

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 33 amino acids, 99 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

158

173

176

N - Asn His Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Cys Ser Thr Cys
 5'- APC CAT CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC TGC AGT ACA TGT
 182 186 190
 Glu Gly Asn Leu Ala Cys Leu Ser Leu Cys His Ile Glu Thr - C
 GAA GGT AAT CTT GCA TGC TTA TCA CTC TGC CAT ATT GAG ACG - 3'

Information for SEQ ID NO: 60 G200V8C

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 61 amino acids, 183 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 200
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu Arg Ala Pro Ser Arg Ala Pro Thr Ile - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA GCA CCA ACA ATC - 3'

Information for SEQ ID NO: 61 G198VδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 59 amino acids, 177 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 198
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu Arg Ala Pro Ser Arg Ala Pro - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA GCA CCA - 3'

Information for SEQ ID NO: 62 G196VδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 57 amino acids, 171 nucleotides
 STRANDEDNESS: single
 TOPOLOGY: linear
 MOLECULE TYPE: protein

140
 N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 196
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu Arg Ala Pro Ser Arg - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA AGA GCA CCA AGC AGA - 3'

Information for SEQ ID NO: 63 G194VδC

SEQUENCE TYPE: amino acids and nucleotides
 SEQUENCE LENGTH: 55 amino acids, 165 nucleotides
 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 194
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu Arg Ala Pro - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA AGA CCA CCA - 3'

Information for SEQ ID NO: 64 G192V8C

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 53 amino acids, 159 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 192
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr Glu Arg - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG GAA AGA - 3'

Information for SEQ ID NO: 65 G6V8C

SEQUENCE LENGTH: 51 amino acids, 153 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

140

N - Leu Pro Ala Thr Arg Lys Pro Pro Ile Asn Pro Ser Gly Ser Ile Pro Pro Glu Asn His
 5'- CTA CCA GCC ACA AGA AAA CCA CCA ATT AAT CCA TCA GGA AGC ATC CCA CCA GAA AAC CAT
 160 173 176
 Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys Glu Gly Asn Leu
 CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT
 182 186 190
 Ala Cys Leu Ser Leu Ser His Ile Glu Thr - C
 GCA TGC TTA TCA CTC AGC CAT ATT GAG ACG - 3'

Information for SEQ ID NO: 66 G7V8C

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 33 amino acids, 99 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: protein

158 173 176
 N - Asn His Gln Asp His Asn Asn Phe Gln Thr Leu Pro Tyr Val Pro Ser Ser Thr Cys
 5'- AAC CAT CAA GAC CAC AAC AAC TTC CAA ACA CTC CCC TAT GTT CCC AGC AGT ACA TGT
182 186 190
 Glu Gly Asn Leu Ala Cys Leu Ser Leu Ser His Ile Glu Thr - C
 GAA GGT AAT CTT GCA TGC TTA TCA CTC AGC CAT ATT GAG AGS - 3'

Information for SEQ ID NO: 67 G4V

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 17 amino acids, 51 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Cys Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Cys His - C
 5'- GTT CCC TGC AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC TGC CAT - 3'

Information for SEQ ID NO: 68 G4VδC

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 17 amino acids, 51 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Ser Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Ser His - C
 5'- GTT CCC AGC AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC AGC CAT - 3'

Information for SEQ ID NO: 69 G4'V

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 17 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Asp Ser Thr Asp Glu Gly Asn Leu Ala Orn Leu Ser Leu Orn His - C

Information for SEQ ID NO: 70 G4'VδC

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 17 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

171 173 176 182 186 187
 N - Val Pro Ser Ser Thr Asp Glu Gly Asn Leu Ala Orn Leu Ser Leu Ser His - C

Information for SEQ ID NO: 71 G1V

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 14 amino acids, 42 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Cys His - C
 5'- AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC TGC CAT - 3'

Information for SEQ ID NO: 72 G1VδC

SEQUENCE TYPE: amino acids and nucleotides

SEQUENCE LENGTH: 14 amino acids, 42 nucleotides

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Thr Cys Glu Gly Asn Leu Ala Cys Leu Ser Leu Ser His - C
 5'- AGT ACA TGT GAA GGT AAT CTT GCA TGC TTA TCA CTC AGC CAT - 3'

Information for SEQ ID NO: 73 G1'VδC

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 14 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

174 176 182 186 187
 N - Ser Thr Asp Glu Gly Asn Leu Ala Orn Leu Ser Leu Ser His - C

Information for SEQ ID NO: 74 G1'

SEQUENCE TYPE: amino acids

SEQUENCE LENGTH: 15 amino acids

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

174 176 182 186 187
N - Ser Ile Asp Ser Asn Asn Pro Thr Orn Trp Ala Ile Ser Lys Cys - C

Claims

- 5 1. Use of at least one fragment of an enterobacterium membrane protein OmpA for preparing a pharmaceutical composition adapted for nasal administration to improve the immunity of a mammal with respect to an antigen or to a hapten.
2. Use of at least one fragment of a membrane protein according to claim 1, characterized in that said enterobacterium membrane is a membrane of *Klebsiella*.
3. Use of at least one fragment of a membrane protein according to claim 2, characterized in that the membrane protein is an OmpA of *Klebsiella pneumoniae*.
- 10 4. Use of at least one fragment of a membrane protein according to one of claims 1 to 3, characterised in that said membrane protein or its fragment is obtained by recombinant process.
5. Use of at least one fragment of a membrane protein according to claim 4, characterized in that said recombinant membrane protein or its fragment is renatured in the presence of detergent chosen from Zwittergent 3-14, Zwittergent 3-12 and octylglucopyranoside.
- 15 6. Use of at least one fragment of a membrane protein according to one of claims 1 to 5, characterized in that at least one fragment has the sequence SEQ ID No 1.
- 20 7. Use according to one of claims 1 to 6, characterized in that the antigen or the hapten are chosen from the group comprising proteins, peptides, polysaccharides, oligosaccharides and nucleic acids.
8. Use of at least one fragment of a membrane protein according to one of claims 1 to 7, characterized in that the antigen or the hapten originate from a virus or from a bacterium.
- 25 9. Use of at least one fragment of a membrane protein according to one of claims 1 to 8, characterized in that the antigen or the hapten comprises at least one protein fragment of a microorganism responsible for pathologies of the airways.

10. Use according to claim 9, characterized in that said microorganism responsible for pathologies of the airways is chosen from RSV, parainfluenzae virus (PIV), influenza virus, hantavirus, streptococci, pneumococci and meningococci.

5 11. Use of at least one fragment of a membrane protein according to one of claims 1 to 10, characterized in that the antigen or the hapten comprises at least one protein fragment of the human or bovine respiratory syncytial virus (RSV).

12. Use according to claim 11, characterized in that the antigen or hapten comprises at least one fragment of the protein G of the RSV.

10 13. Use according to either of claims 11 and 12, characterized in that the antigen or the hapten comprises at least one of the sequences SEQ ID No 2 to SEQ ID No 74.

14. Use according to one of claims 1 to 13, characterized in that said fragment of a membrane protein is covalently coupled to said antigen or hapten.

15 15. Use according to claim 14, characterized in that one or more bonding elements is introduced into the fragment of membrane protein and/or of the antigen or of the hapten in order to facilitate the coupling.

16. Use according to claim 15, characterized in that the bonding element introduced is an amino acid.

20 17. Use according to claim 14, characterized in that the hybrid protein, which is obtained after coupling between the fragment of a membrane protein and the antigen or the hapten, when said antigen or hapten is protein in nature, is prepared by genetic recombination.

25 18. Use according to one of claims 14 to 17, characterized in that the pharmaceutical composition contains a fragment of a membrane protein coupled to an antigen or a hapten.

19. Use according to claim 17, characterized in that the pharmaceutical composition contains a transformed host cell which is capable of expressing a

hybrid protein containing said fragment of membrane protein coupled to said antigen or hapten.

20. Use according to either of claims 18 and 19, characterized in that the pharmaceutical composition does not contain any adjuvant.

5 21. Method for preparing a protein or one of its fragments by recombinant process, characterized in that said protein or one of its fragments is, after extraction, renatured in the presence of a solution comprising a detergent chosen from Zwittergent 3-14, Zwittergent 3-12 and octylglucopyranoside, and in that said recombinant protein is an enterobacterium membrane protein OmpA.

10 22. A use according to claim 1, substantially as described herein with reference to the Examples.

23. A method of improving the immunity of a mammal with respect to an antigen or to a hapten comprising nasally administering at least one fragment of an enterobacterium membrane protein OmpA to the mammal.

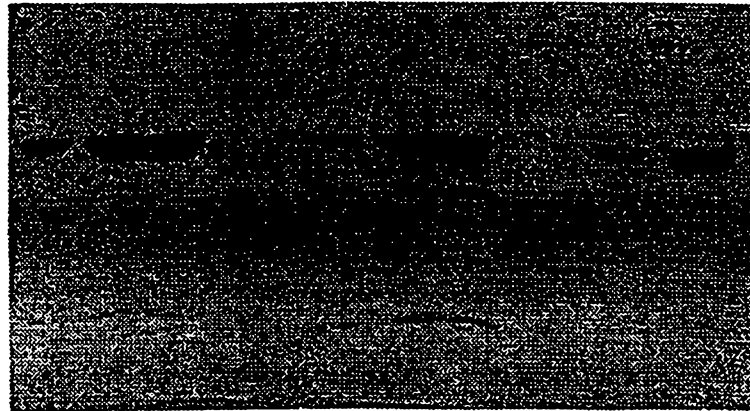
15 24. A method according to claim 23 substantially as described herein with reference to the Examples.

25. A method according to claim 21 substantially as described herein with reference to the Examples.

20 **Dated this 5th day of June 2003**

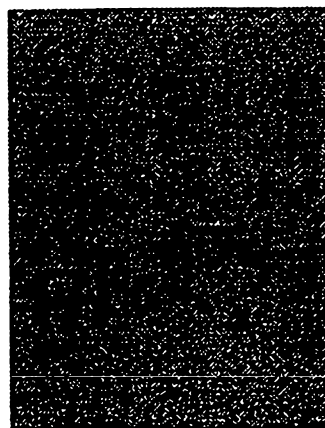
Pierre Fabre Medicament
by its attorneys
Freehills Carter Smith Beadle

25



1 2 3 4 5 6

FIGURE 1A



Std 1 2

FIGURE 1B

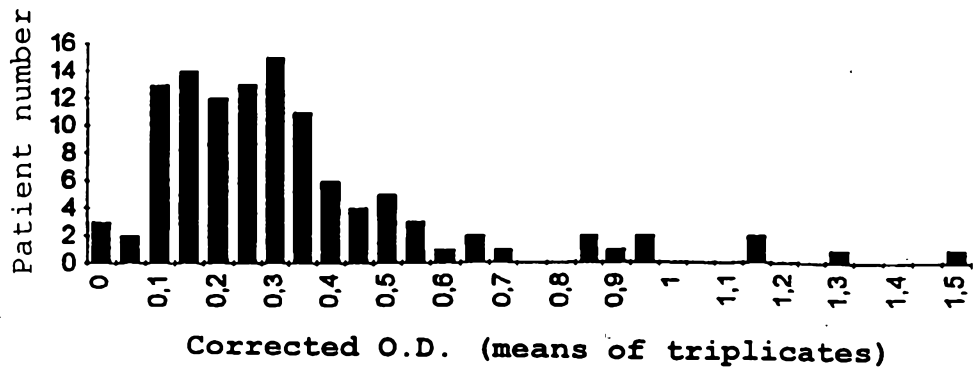


FIGURE 2

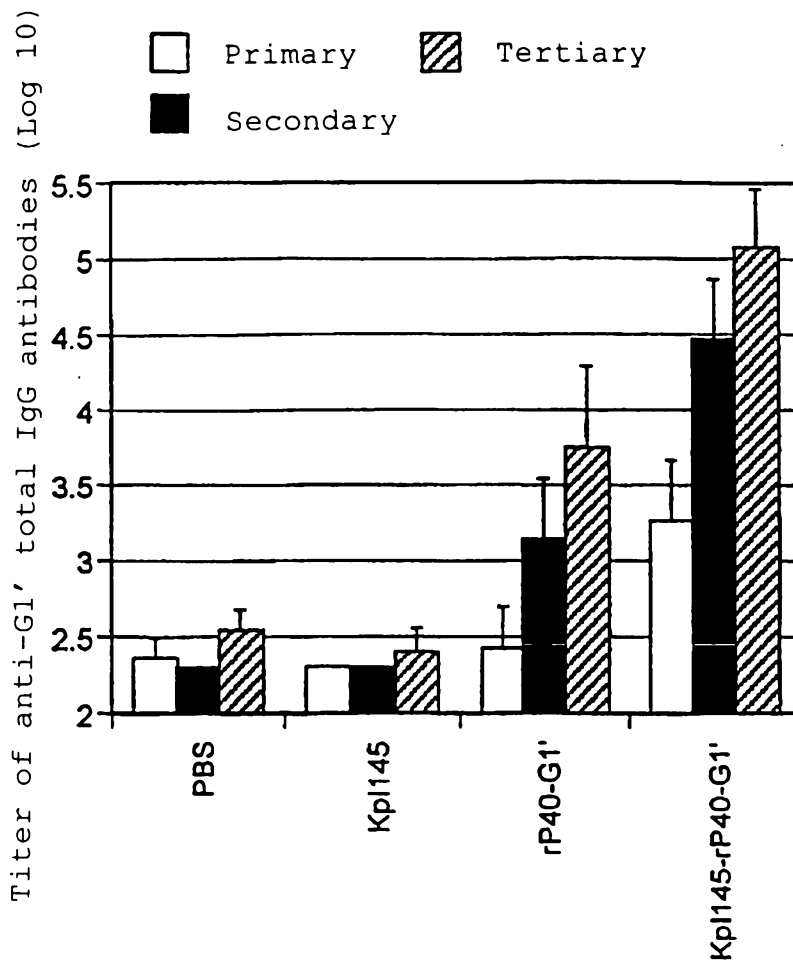


FIGURE 3

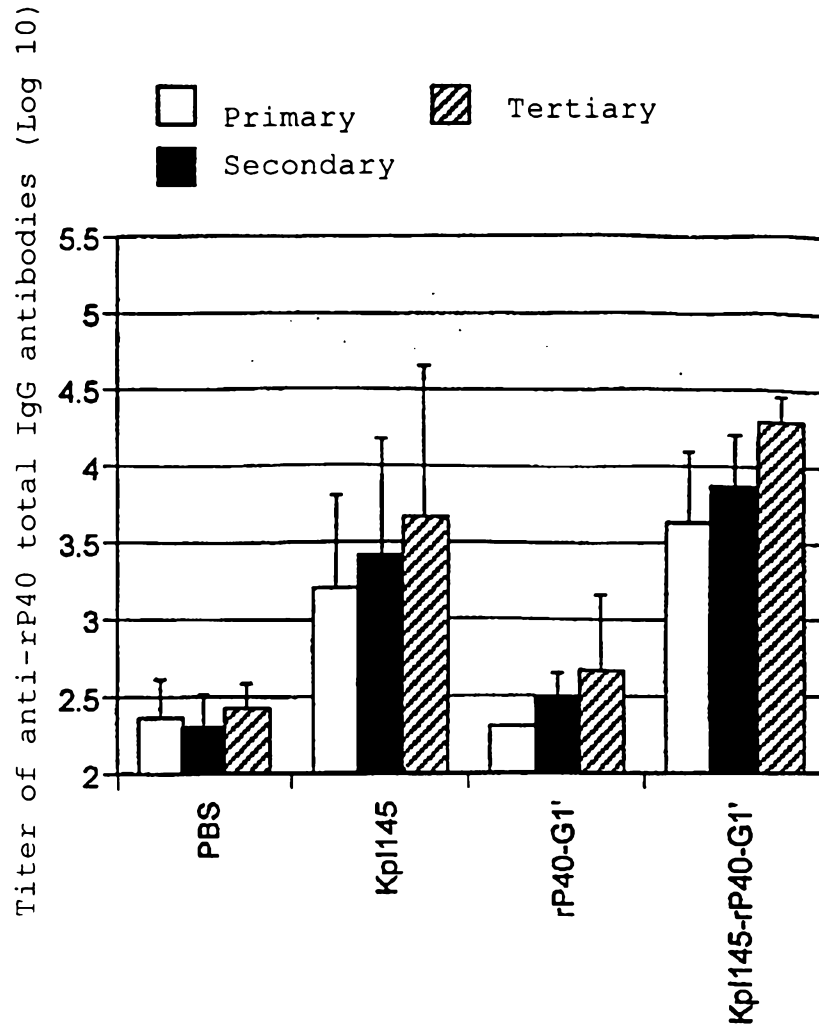


FIGURE 4

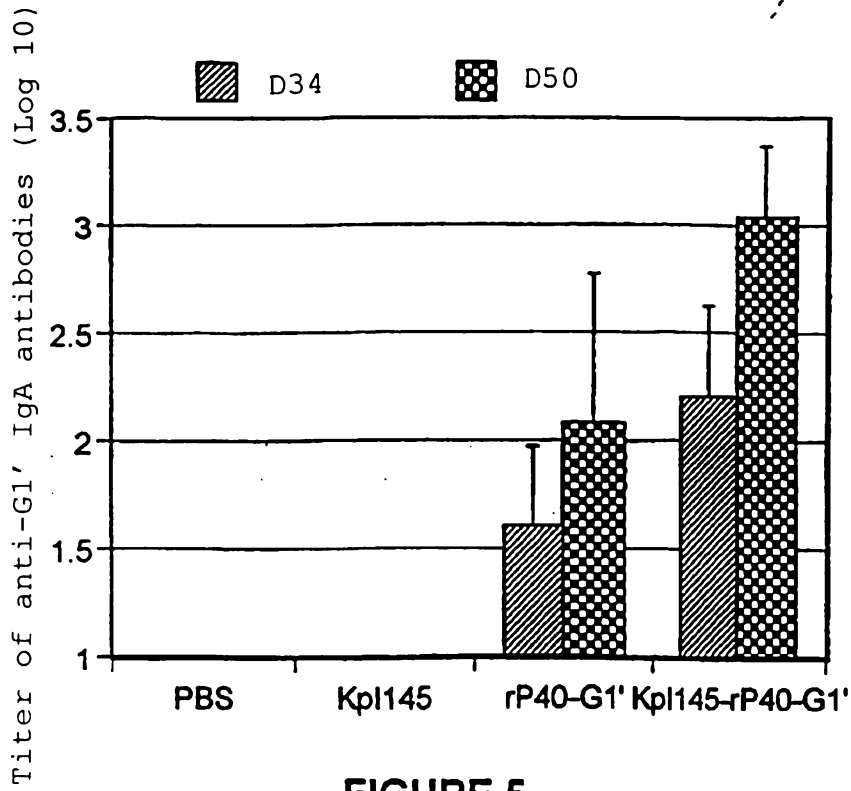


FIGURE 5

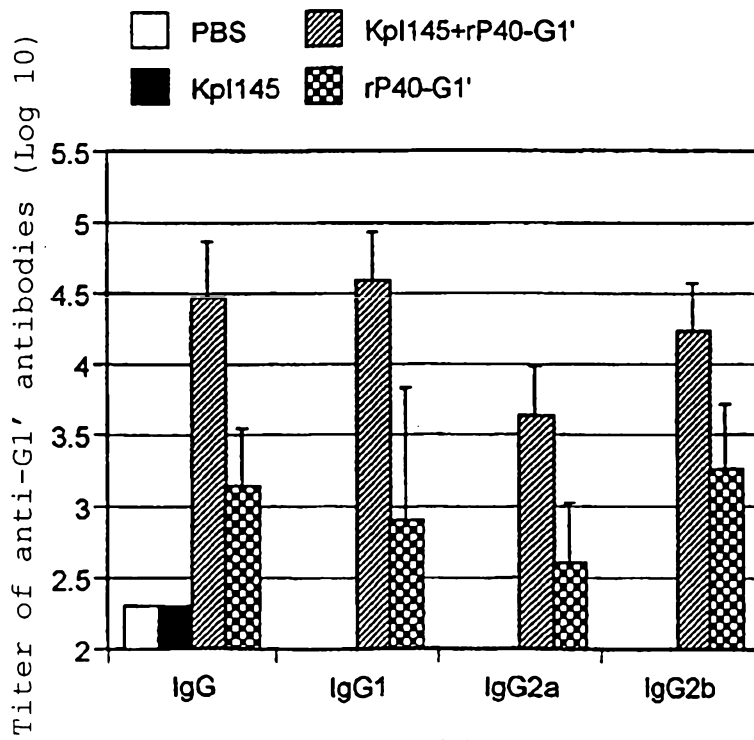


FIGURE 6

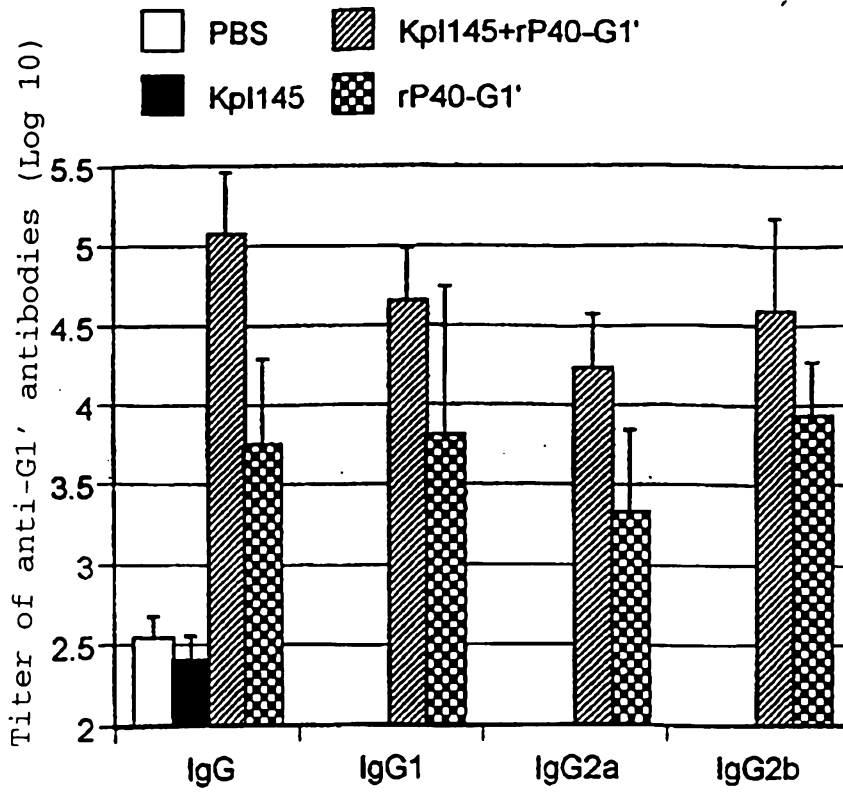


FIGURE 7

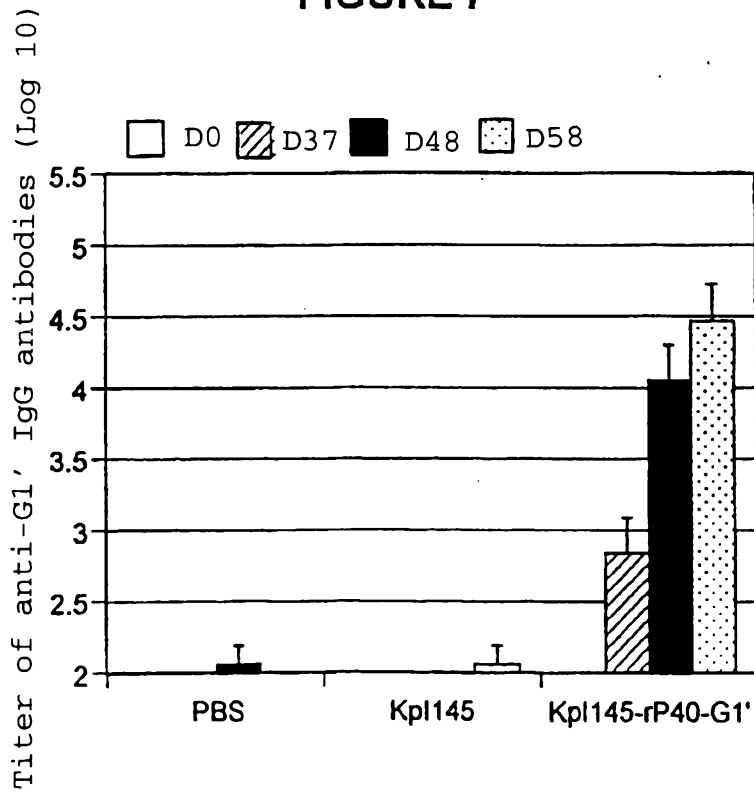


FIGURE 8

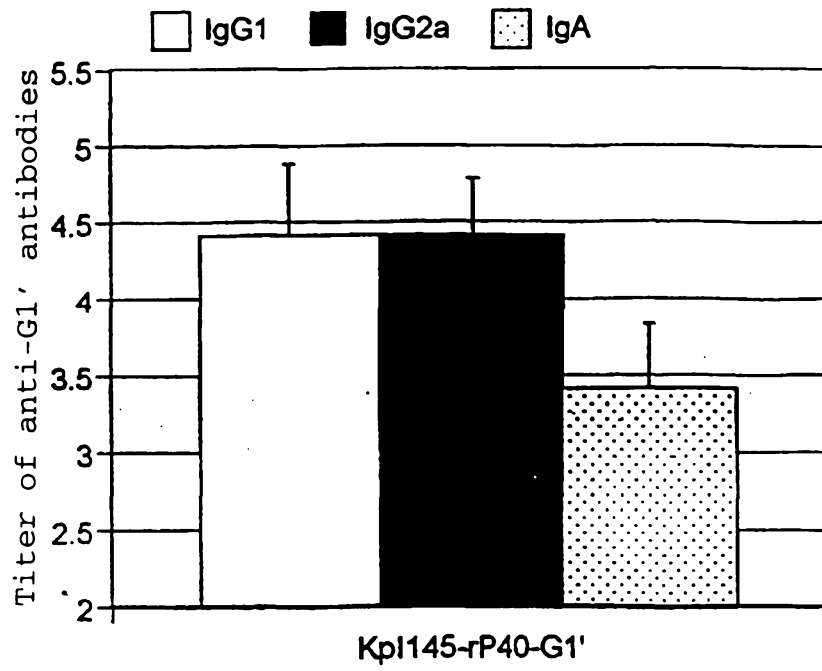


FIGURE 9

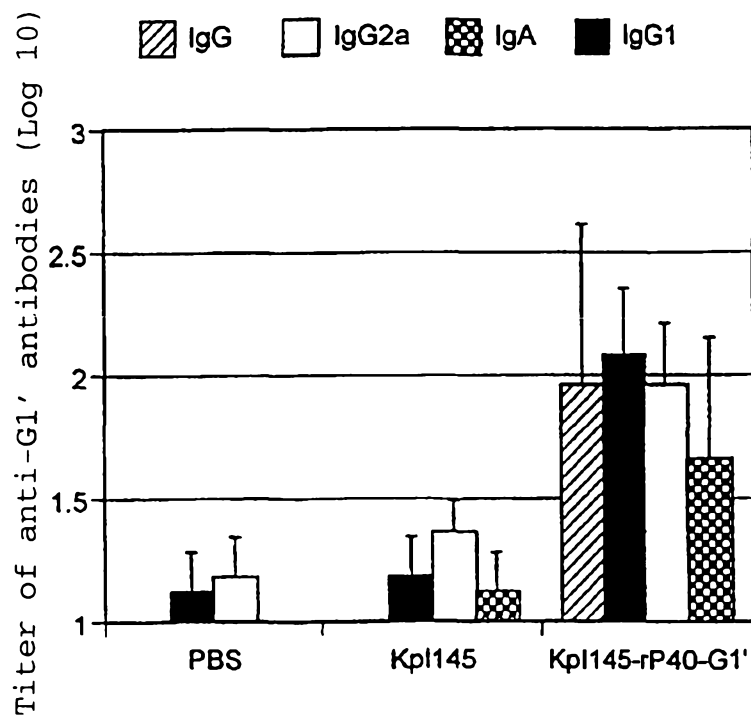


FIGURE 10