

02 112914

PCT-PATENT

Radicación : 02112914 00000000

Folios: 102

Fecha (RD): 2002-12-12 16:50:10

Trámite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTA

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

ción

**FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL**

1

SOLICITUD DE:



Patente de Invención.



Patente de Modelo de Utilidad.



Capítulo I.



Capítulo II.

2

SOLICITANTE
(71)Nombre: **UNIVERSITY OF
SASKATCHEWAN**sociedad constituida y existente bajo las
leyes de Canadá.Dirección: 120 Veterinary Road,
Saskatoon, Saskatchewan, S7N 5E3,
Canadá E3Domicilio: Saskatoon, Saskatchewan.
Canadá.

IDENTIFICACION

C.C.



NIT



C.E.



Otro



Cual

Número:

3

REPRESENTANTE
O APODERADONombre: **EMILIO FERRERO**

Dirección: Carrera 4ª. No. 72-35

Teléfono: 347 36 11

Fax:: 217 82 11

E-mail: clientes@camyo.com.

Domicilio: Bogotá, Colombia.

IDENTIFICACION

C.C.



NIT



C.E.



Otro



Cual

Número: 438.019 T.P. 31.929

4

INVENTOR (ES)
(72)

1- Andrew A. POTTER

521 Dalhousie Crescent,

Saskatoon, Saskatchewan S7H 3S5,

Canadá.

3- Xin Ming SONG

8 1506 Main Street

Saskatoon, Saskatchewan S7H 0L7

Canadá.

2- Alexandra J. BOLTON

115-1540 29th Street N.W.

Calgary, Alberta T2N 4M1

Canadá

5

PCT (86)

Solicitud Internacional No. PCT/CA01/00837Publicación Internacional No. WO 01/86380 A2Fecha: 11 de junio 2001Fecha : 20 de diciembre de 2001

6

Título (54) INMUNIZACION DE GANADO LECHERO CON PROTEINA Mlg

7

Clasificación Internacional (51) C07K 14/195A61K 39/09

8

Prioridad

SI



NO



(33) País de Origen

US.

(32) Fecha

12 de junio de 20022000

(31) Número de Solicitud

60/211,018

9

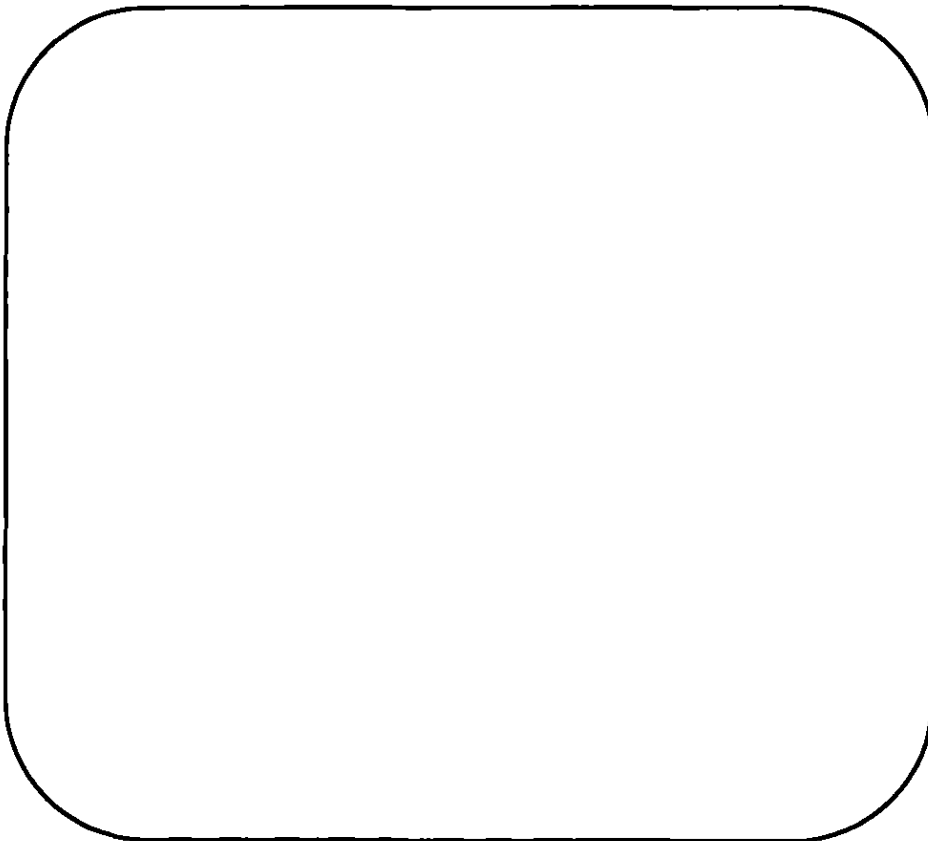
Comprobante de pago No.: 02-62.546 Fecha: 9 de diciembre de 2002

10

Anexos:

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 378.000.00.
- ☐ Comprobante de pago por reivindicación de prioridad.
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☐ Traducción de la solicitud Internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
- ☐ Traducción del examen preliminar efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ Otros

11

FIGURA CARACTERISTICA:

12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERRERO

FIRMA : 
C.C. 438.019 de Usaquén T.P. 31.929

JBP/mrm-ld-2-



Presentation: Front

Image: Small

Français



1 of 1

[View Images](#)

PCT

World Intellectual Property Organization
International Bureau(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT
COOPERATION TREATY (PCT)(51) International Patent Classification⁷
C07K 14/195

A2

(11) International Publication Number WO
01/96380(43) International Publication Date 20
December 2001
(20.12.2001)

(21) International Application Number PCT/CA01/00837

(22) International Filing Date 11 June 2001
(11.06.2001)

(30) Priority Data

60/211,016 12 June 2000 (12.06.2000) US

(71) Applicant:

UNIVERSITY OF SASKATCHEWAN 120
Veterinary Road, Saskatoon, Saskatchewan S7N
5E3; (CA). [CA/CA].

(72) Inventors

POTTER, Andrew, A. 521 Dalhousie Crescent,
Saskatoon, Saskatchewan S7H 3S5; (CA).
BOLTON, Alexandra, J. BOLTON, Alexandra,
J.; (CA). SONG, Xin, Ming SONG, Xin, Ming;
(CA).

(74) Agent

ERRATT, Judy, A. Gowlings Lafleur Henderson
LLP, Suite 2600, 160 Elgin Street, Ottawa, Ontario
K1P 1C3; (US).(81) Designated States
(national)

CA, CO

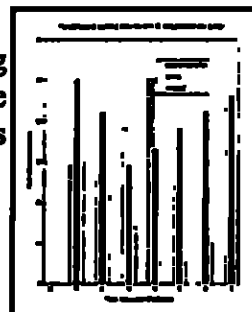
(84) Designated States
(regional)European patent (AT,
BE, CH, CY, DE, DK,
ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE,
TR)For information on time limits
for entry into the national
phase please click here

Published

*without international
search report and to be
republished upon receipt
of that report*

(54) Title IMMUNIZATION OF DAIRY CATTLE WITH Mig PROTEIN

(57) Abstract

The Mig protein gene of *Streptococcus dysgalactiae*, and the corresponding amino acid sequence, is described, as is the use of the Mig protein in vaccine compositions to prevent and treat bacterial infections in general, and mastitis in particular.

Presentation: Front

Image: Small

Français



1 of 1

IMMUNIZATION OF DAIRY CATTLE WITH M1S PROTEIN TECHNICAL FIELD

The present invention relates generally to bacterial antigens and genes encoding the same. More particularly, the present invention pertains to the cloning, expression and characterization of the M1g Fc-receptor protein from several *Streptococcus* bacteria species, and the use of the same in vaccine compositions.

BACKGROUND Mastitis is an infection of the mammary gland usually caused by bacteria or fungus.

The inflammatory response following infection results in decreased milk yield as well as quality, and causes major annual economic losses to the dairy industry.

Among the bacterial species most commonly associated with mastitis are various species of the genus *Streptococcus*, including *Streptococcus aureus*, *Streptococcus uberis* [(UNTYPEABLE),] *Streptococcus agalactiae* (Lancefield group [B],) *Streptococcus dysgalactiae* (Lancefield group C), [STREPTOCOCCUS ZOOEPIDEMICUS, AND] the Lancefield groups D, G, L and N streptococci. Some of those species are contagious (e. g. *S. agalactiae*), while others are considered environmental pathogens (e. g. *S. [DYS GALACTIAE]* and *S. [UBERIS]*.)

The environmental pathogen *S. uberis* is responsible for about 20% of all clinical cases of mastitis (Bramley, A. J. [AND DODD,] F. H. (1984) [J : DY. 51] : 481-512 ; Bramley, A. J. (1987) [ANIMAL HEALTH NUTRITION 42] : 12-16; Watts, J. L. (1988) J. Dairy Sci. 71: 1616-1624); it is the predominant organism isolated from mammary glands during the non-lactating period (Bramley, A. J. (1984) Br. Vet. [J] 140: 328-335 ; Bramley and Dodd (1984) [J] [D. 51] : 481-512 ; Oliver, S. P. (1988) Am. J. Vet. Res. 49: 1789-1793).

Mastitis resulting from infection with *S. [UBERIS]* is commonly [SUBCLINICAL,] characterized by apparently normal milk with an increase in somatic cell counts due to the influx [OF LEUKOCYTES.]

The chemical composition of milk is changed due to suppression of secretion with the transfer of sodium chloride and bicarbonate from blood to milk, causing a shift of pH to a more alkaline level. *S. uberis* mastitis may also take the form of an acute clinical

condition, with obvious signs of disease such as clots or discoloration of the milk and swelling or hardness of the mammary gland. Some cases of the clinical disease can be severe and pyrexia may be present. For a review of the clinical manifestations [OF *S. UBERIS*] mastitis, see, Bramley (1991) Mastitis: physiology or pathology. p. 3-9. In C. Burvenich, G.

[VANDEPUTTE-VAN] Messom, and A. W. Hill (ed.), New insights into the pathogenesis of mastitis. [LLIJKSUNIVERSITEIT] Gent, Belgium ; and Schalm et al. (1971) The mastitis complex-A brief summary. p. [1-3.] In Bovine Mastitis. Lea & Febiger, Philadelphia.

Conventional antibacterial control methods such as teat dipping and antibiotic therapy are effective in the control of many types of contagious mastitis, but the environmental organisms typically found in all dairy barns are often resistant to such measures. Vaccination is therefore an attractive strategy to prevent infections of the mammary glands, and has been shown to be beneficial in the case of some contagious mastitis pathogens.

However, the literature is limited regarding vaccination studies with environmental pathogens such as *S. dysgalactiae* and [*S. UBERIS*,] and variable results have been observed. In some cases, immunization has resulted in increased sensitivity to the specific organism and in other cases strain-specific protection has been obtained.

For example, previous studies have shown that primary infection with *S. uberis* can considerably reduce the rate of infection following a second challenge with the same strain (Hill, A. W. (1988) Res. Vet. Sci. 44: [386-387].) Local vaccination with killed *S. [UBERIS]* protects the bovine mammary gland against intramammary challenge with the homologous strain (Finch et al. (1994) Infect. Immun. 62: 3599-3603). Similarly, subcutaneous vaccination with live *S. uberis* has been shown to cause a dramatic modification of the pathogenesis of mastitis with the same strain (Hill et al. (1994) [FEMS IMMUNOL. MED.]

[MICROBIOL. 8 :] 109-118). Animals vaccinated in this way shed fewer bacteria in their milk and many quarters remain free of infection. Nonetheless, vaccination with live or attenuated bacteria can pose risks to the recipient. Further, it is clear that conventional killed vaccines are in general largely ineffective against *S. uberis* and *S. agalactiae*, either due to lack of protective antigens on in vitro-grown cells or masking of these antigens by molecular mimicry.

6

The current lack of existing mastitis vaccines against *S. agalactiae* or the contagious streptococcus strains is due at least in part to a lack of knowledge regarding the virulence determinants and protective antigens produced by those organisms which are involved in invasion and protection of the mammary gland (Collins et al. (1988) [J DAIRY RES.] 55: 25-32; Leigh et al. (1990) Res. Vet. Sci. 49: 85-87 ; Marshall et al. (1986) [J. DAIRY RES. 53] : 507-514). *S. dysgalactiae* is known to bind several extracellular and plasma-derived proteins such as fibronectin, fibrinogen, collagen, [ALPHA-II-MACROGLOBULIN,] IgG, albumin and other compounds. The organism also produces hyaluronidase and fibrinolysin and is capable of adhering to and invading bovine mammary epithelial cells. However, the exact roles of the bacterial components responsible for these phenotypes in pathogenesis is not known. Similarly, the pathogenesis of *S. uberis* infection is poorly understood. Furthermore, the influence [OF *S. UBERIS*] virulence factors on host defense mechanisms and mammary gland physiology is not well defined. Known virulence factors associated with *S. uberis* include a hyaluronic acid capsule (Hill, A. W. (1988) Res. Vet. Sci. 45: 400-404), hyaluronidase (Schaufuss et al. (1989) [ZENTRALBL. BAKTERIOL. SER. A 271] : 46-53), R-like protein (Groschup, M. H. and Timoney, J. F. (1993) Res. Vet. Sci. 54: 124-126), and a cohemolysin, the CAMP factor, also known as [UBERIS] factor (Skalka, [B.] and Smola, J. [(1981) ZENTRALBL. BAKTERIOL.] Ser. A 249: 190-194), [R-LIKE] protein, plasminogen activator and CAMP factor. However, very little is known of their roles in pathogenicity.

The use of virulence determinants from *Streptococcus* as immunogenic agents has been proposed. For example, the CAMP factor of [*S. UBERIS*] has been shown to protect vertebrate subject from infection by that organism (Jiang, et al., U. S. Patent No. 5,863,543). The [Y] antigen of the group B *Streptococci* strain A909 (ATCC No. 27591) is a component of the c protein marker complex, which additionally comprises an a and [P] subunit (Boyle, U. S. Patent No. 5,721,339). Subsets of serotype [LA,] II, and virtually all serotype Ib cells of group B streptococci, have been reported to express components of the c protein. Use of the [Y] subunit as an immunogenic agent against infections by Lancefield Group B *Streptococcus* infection has been proposed. However, its use to prevent or treat bacterial infections in animals, including mastitis in cattle, has not been studied.

The group A streptococcal M protein is considered to be one of the major virulence factors of this organism by virtue of its ability to impede attack by human phagocytes (Lancefield, R. C. (1962) J. Immunol. 89: 307-313). The bacteria persist in the infected tissue until antibodies are produced against the M molecule. Type-specific antibodies to the M protein are able to reverse the antiphagocytic effect of the molecule and allow efficient clearance of the invading organism.

M proteins are one of the key virulence factors of *Streptococcus pyogenes*, due to their involvement in mediating resistance to phagocytosis (Kehoe, M. A. [(1991) VACCINE] 9: 797-806) and their ability to induce potentially harmful host immune responses via their superantigenicity and their capacity to induce host-cross-reactive antibody responses (Bisno, A. L. (1991) New Engl. J. Med. 325: 783-793; Froude et al. (1989) Curr. Top. [MICROBIOL.] Immunol. 145: [5-26] ; [STOLLERMAN,] G. H. (1991) [CLIN. IMMUNOL. IMMUNOPATHOL.] 61: 131-142).

However, obstacles exist to using intact M proteins as vaccines. The protein's opsonic epitopes are extremely type-specific, resulting in narrow, type-specific protection. Further, some M proteins appear to contain epitopes that cross react with tissues of the immunized subject, causing a harmful autoimmune response (See e. g. Dale, J. G. and Beachey, E. H. (1982) [R] Exp. [MED 156] : 1165-1176; Dale, J. B. and Beachey, E. H. [(1985)] [A] [EXP.] Med. 161: 113-122 ; Baird, R. W., Bronze, M. S., Draus, W., Hill, H. R., Veasey, L. G. and Dale, J. B. (1991) [J IMMUN. 146] : [3132-3137 ;] Bronze, M. S. and Dale, J. B. (1993) J Immun 151: 2820-2828; Cunningham, M. W. and Russell, S. M. (1983) Infect. Immun. 42: [531-538].)

Chimeric proteins containing three different fibronectin binding domains [(FNBDs)] derived from fibronectin binding proteins of *S. dysgalactiae* and *Staphylococcus aureus* have been expressed on the surface of [STAPH.] [CARNOSUS] cells. In the case of one of these

7

proteins, intranasal immunizations with live recombinant [STAPH.] [CARNOSUS] cells expressing the chimeric protein on their surface resulted in an improved antibody response to a model immunogen present within the chimeric surface protein (Liljeqvist, S. et al. (1999) FEBS Letters 446: 299-304).

Bacterial Fc receptors (surface moieties that bind to immunoglobulin molecules through a non-immune mechanism, i. e., to the Fc portion of the antibody) are a class of binding proteins further categorized by their reactivity with different classes and subclasses of mammalian immunoglobulins. The type I receptor (also known as Protein A), the most extensively studied and characterized, has been isolated from [STAPYLOCOCCUS AUREUS,] and binds to IgG types 1,2 and 4; this receptor type further exhibits cross-reactivity with IgA and IgM. The type II Fc receptor, found on a few Lancefield Group A streptococci, and the type III receptor (also known as Protein G), found on the majority of human group C and group G strains of streptococcus, have been reported to react with all four types of IgG. In the case of the type III receptor, binding to IgG is highly specific; the protein does not cross-react with IgA or IgM. The type IV receptor is found in certain bovine group G streptococci, and the type V receptor is found on certain strains [OF STREPTOCOCCUS ZOOEPIDEMICUS.] The type VI Fc receptor has been isolated from S. [ZOOEPIDEMICUS] strains [S212] and RSS-212, and binds rat IgG with high affinity, i. e., 100 times that of Protein A binding, and 30 to 40 times as great as Protein G binding. (Boyle, et al., U. S. Patent No. 4,977,082). For a discussion of Fc receptors, see Langone (1982) Adv. Immunol. 32: 167 and Myhre et al. (1984) Basic Concepts of Streptococci and Streptococcal Diseases (Holm & Christensen, eds.) Redbook Ltd., Chertsey, Surrey, England. Utility for Fc binding proteins have to date been limited to antibody detection and purification. With respect to clinical applications, [A] method of extracorporeal blood treatment of autoimmune disease which employs Fc binding proteins to remove antigen- antibody complexes has been proposed (see e. g. Fahnestock, U. S. Patent No. 4,954,618).

However, their use in vaccine compositions has not previously been described nor suggested.

Until now, the protective capability of the S. dysgalactiae Mig protein against mastitis has not been studied, nor has the S. dysgalactiae Mig protein been isolated or characterized.

SUMMARY OF THE INVENTION Accordingly, the present invention provides Fc receptor proteins and uses therefor.

In one embodiment, the invention is directed to a vaccine composition comprising a pharmaceutically acceptable vehicle and an Fc receptor protein. In certain embodiments, the Fc receptor protein is selected from the group consisting of: (a) a Streptococcus dysgalactiae Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO: 4); (b) an Fc receptor protein having at least about 70% sequence identity to (a); and (c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids. In some embodiments, the vaccine composition comprises and adjuvant.

In yet further embodiments, the invention is directed to a method of producing a vaccine composition. The method comprises the steps of (a) providing an Fc receptor protein or an immunogenic fragment thereof, the fragment comprising at least about 5 amino acids, and (b) combining said protein with a pharmaceutically acceptable vehicle.

In another embodiment, the invention is directed to a method of treating or preventing a bacterial infection in a vertebrate subject. The method comprises administering to the subject a therapeutically effective amount of a vaccine composition as described above.

In certain embodiments the bacterial infection is a streptococcal infection.

Additionally, the bacterial infection may cause mastitis.

In yet another embodiment, the invention is directed to a method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to the subject a therapeutically effective amount of a polynucleotide that comprises a coding sequence for an Fc receptor protein.

In certain embodiments, the bacterial infection is a streptococcal infection and may cause mastitis.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the

disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES Figures [1A-1D] depict the polynucleotide sequence encoding the *Streptococcus dysgalactiae* Mig protein, and the amino acid sequence deduced therefrom [(SEQ ID NO] : 3 and SEQ ID NO : 4, respectively).

Figure 2 presents the following results for peptide structure analysis of the *S. dysgalactiae* Mig protein a: Kyle-Doolittle Hydropathy plot ("KD Hydrophilicity"), averaged over a window of 7; an Emini surface probability plot ("Surface Prob."); a Karplus-Schulz chain flexibility plot ("Flexibility"); a [JAMESON-WOLF ANTIGENIC] index plot, and both Chou- Fasman and [GARNIER-OSGUTHORPE-ROBSON] secondary structure plots ("CF Alpha Helices" and "CF Beta Sheets" ; and "GOR Turns," "GOR Alpha Helices," "GOR Beta Sheets," and "Glycosylation Sites," respectively). Figure 3 is a Chou-Fasman secondary structure plot for the *S. dysgalactiae* Mig protein.

Figure 4 compares the change in the percentage of udder quarters infected with *S. dysgalactiae* over a 7 day period among three experimental groups: (1) unvaccinated control animals; (2) animals vaccinated with the Mig protein ; and (3) animals vaccinated with GapC, a plasmin binding protein isolated from *S. dysgalactiae* that was simultaneously evaluated.

Infection was defined as recovery [OF >500 CFU OF THE *S. DYSGALACTIAE*] per ml of milk secretions.

Figure 5 depicts the observed inflammatory response to infection with *S. dysgalactiae* plotted as mean somatic cell counts (SCC) for each experimental group versus time in days post challenge. In the figure, diamonds [(-+)] represent unchallenged, unvaccinated quarters, squares [(-M)] represent challenged, unvaccinated animals, triangles [(-A)] represent challenged, Mig-vaccinated animals, and x's [(-X)] represent challenged, GapC-vaccinated animals.

Figure 6 illustrates somatic cell counts per mammary quarter on day 1 post-challenge.

In the figure, the bar represents the mean for each group. Squares [(-)] represent unvaccinated animals; triangles [(-c)] represent GapC-vaccinated animals, and inverted triangles represent Mig-vaccinated animals.

DETAILED DESCRIPTION OF THE INVENTION The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e. g., Sambrook, Fritsch & Maniatis, *Molecular Cloning : A Laboratory Manual*, Vols. [I,] II and III, Second Edition (1989); Perbal, B., *A Practical Guide to Molecular Cloning* [CLONING] (1984); the series, *Methods In ENZYMOLOGY* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. [I-IV] (D. M. Weir and C. C. Blackwell eds., 1986, [BLACKWELL] Scientific Publications).

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A) Arginine: Arg (R) Asparagine: Asn (N) Aspartic acid: Asp (D) Cysteine: Cys (C) Glutamine: Gln (Q) Glutamic acid: Glu (E) Glycine: Gly (G) Histidine: His (H) Isoleucine: [ILE] [(I)] Leucine: Leu (L) Lysine: Lys (K) Methionine: Met (M) Phenylalanine : Phe (F) Proline: Pro (P) Serine: Ser (S) Threonine: [TLIR] (T) Tryptophan: Trp [(W)] Tyrosine: Tyr (Y) Valine: Val (V) 1.

DEFINITIONS In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a *Streptococcus* [DYSGALACTIAE] Fc binding protein" includes a mixture of two or more such proteins, and the n The terms "Fc receptor [PROTEIN AND Fc] binding protein," used interchangeably herein, denote a bacterial protein capable of binding to immunoglobulin molecules at a site other than the antigen recognition site, including without limitation the Fc region of an immunoglobulin molecule.

The terms "Mig protein" and "Mig Fc receptor protein" and "Mig Fc binding protein" (used interchangeably herein) or a nucleotide sequence encoding the same, intends a protein or a nucleotide sequence, respectively, which is derived from a Mig gene found in a variety of bacterial species, including, without limitation, certain strains of group A streptococci.

The nucleotide sequence of a representative *Streptococcus* Mig gene from *S. [DYS GALACTIAE]* (SEQ ID NO : 3), and the corresponding amino acid sequence encoded by that gene (SEQ ID NO : 4), is depicted in Figures [LA-1D.] However, a Mig protein as defined herein is not limited to the depicted sequences as subtypes of each of these *Streptococcus* species are known and variations in Mig proteins will occur between them.

A representative Mig gene derived from [*S.*] *dysgalactiae* is found in the plasmid [PAA505MIG.]

Furthermore, the derived protein or nucleotide sequences need not be physically derived from the gene described above, but may be generated in any manner, including for example, chemical synthesis, isolation (e. g., from *S. dysgalactiae*) or by recombinant production, based on the information provided herein. Additionally, the term intends proteins having amino acid sequences substantially homologous (as defined below) to contiguous amino acid sequences encoded by the genes, which display immunological and/or plasmin-binding activity.

Thus, the terms intend full-length, as well as immunogenic, truncated and partial sequences, and active analogs and precursor forms of the proteins. Also included in the term are nucleotide fragments of the gene that include at least about 8 contiguous base pairs, more preferably at least about 10-20 contiguous base pairs, and most preferably at least about 25 to 50, or more, contiguous base pairs of the gene, or any integers between these values. Such fragments are useful as probes and in diagnostic methods, discussed more fully below.

The terms also include those forms possessing, as well as lacking, a signal sequence, if such is present, as well as the nucleic acid sequences coding therefore. Additionally, the term intends forms of the Mig proteins which lack a membrane anchor region, and nucleic acid sequences encoding proteins with such deletions. Such deletions may be desirable in systems that do not provide for secretion of the protein. Furthermore, the Fc receptor- binding domains of the proteins, may or may not be present. Thus, for example, if the Mig Fc binding protein will be used to purify immunoglobulin, the Fc binding domain will generally be retained. If the protein is to be used in vaccine compositions, immunogenic epitopes which may or may not include the Fc receptor binding domain, will be present.

The terms also include proteins in neutral form or in the form of basic or acid addition salts depending on the mode of preparation. Such acid addition salts may involve free amino groups and basic salts may be formed with free carboxyls. Pharmaceutically acceptable basic and acid addition salts are discussed further below. In addition, the proteins may be modified by combination with other biological materials such as lipids (both those occurring naturally with the molecule or other lipids that do not destroy immunological activity) and saccharides, or by side chain modification, such as acetylation of amino groups, phosphorylation of hydroxyl side chains, oxidation of sulfhydryl groups, glycosylation of amino acid residues, as well as other modifications of the encoded primary sequence.

The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein.

In this regard, particularly preferred substitutions will generally be conservative in nature, i. e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate ; (2) basic [--LYSINE,] arginine, histidine ; (3) non-polar--alanine, valine, leucine, isoleucine, proline, [PHENYLALANINE,] methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cystine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa ; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule, but possessing minor amino acid substitutions that do not substantially affect the immunogenicity and/or plasmin-binding affinity of the protein, are therefore within the definition of the reference polypeptide.

For example, the polypeptide of interest may include up to about

5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 or 20-50 conservative or non-conservative amino acid substitutions, or any integer between these values, so long as the desired function of the molecule remains intact. Similarly, substitutions occurring in the transmembrane binding domain, if present, and the signal sequence, if present, normally will not affect immunogenicity. One of skill in the art may readily determine other regions of the molecule of interest that can tolerate change by reference to the peptide structure plots shown in Figure 2 and Figure 3 herein.

The [TERM"STREPTOCOCCAL] Mig protein"intends a Mig Fc binding protein, as defined above, derived from a streptococcal species that produces the same, including, but not limited to *S. dysgalactiae*. For example, a "S. dysgalactiae Mig protein" is a Fc binding protein as defined above, derived from *S. dysgalactiae*.

"Wild type" or "native" proteins or polypeptides refer to proteins or polypeptides isolated from the source in which the proteins naturally occur. "Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques ; i. e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

An "isolated" protein or polypeptide is a protein or polypeptide molecule separate and discrete from the whole organism with which the molecule is found in nature; or a protein or polypeptide devoid, in whole or part, of sequences normally associated with it in nature ; or [A] sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith.

The [TERM"FUNCTIONALLY] equivalent" intends that the amino acid sequence of a Mig Fc binding protein is one that will elicit a substantially equivalent or enhanced immunological response, as defined above, as compared to the response elicited by a Fc binding protein having identity with the reference Fc binding protein, or an immunogenic portion thereof.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

The [TERMS"IMMUNOGENIC"PROTEIN] or polypeptide refer to an amino acid sequence which elicits an immunological response as described below. [AN"IMMUNOGENIC"PROTEIN] or polypeptide, as used herein, includes the full-length sequence of the Fc receptor protein in question, with or without the signal sequence, membrane anchor domain and/or Fc binding domain, analogs thereof, or immunogenic fragments thereof.

[BY"IMMUNOGENIC] fragment" is meant a fragment of an Fc receptor protein which includes one or more epitopes and thus elicits the immunological response described below.

Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e. g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e. g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e. g., U. S. Patent No. 4,708,871 ; Geysen et al. (1984) [PROC.] [NATL. ACAD. SCI. USA] 81: [3998-4002 ;] Geysen et al. (1986) Molec. Immunol. 23: 709-715.

Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e. g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e. g., [EPITOPE MAPPING PROTOCOLS, SUPRA.] Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots, such as those calculated using, e. g., the Omega version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al., Proc. Natl. Acad. Sci USA (1981) 78: [3824-3828] for determining antigenicity profiles, and the Kyte-Doolittle technique, Kyte et al., [J MOL. BIOL.] (1982) 157: 105-132 for hydropathy plots. Figures 2 and 3 herein depict Kyte-Doolittle profiles for representative proteins encompassed by the invention.

Immunogenic fragments, for purposes of the present invention, will

usually include at least about 3 amino acids, preferably at least about 5 amino acids, more preferably at least about 10-15 amino acids, and most preferably 25 or more amino acids, of the parent Mig Fc receptor protein molecule. There is no critical upper limit to the length of the fragment, which may comprise nearly the full-length of the protein sequence, or even a fusion protein comprising two or more epitopes of Mig.

An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

[BY "SUBUNIT] vaccine composition" is meant a composition containing at least one immunogenic polypeptide, but not all antigens, derived from or homologous to an antigen from a pathogen of interest. Such a composition is substantially free of intact pathogen cells or particles, or the lysate of such cells or particles. Thus, a "subunit vaccine composition" is prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or recombinant analogs thereof. A subunit vaccine composition can comprise the subunit antigen or antigens of interest substantially free of other antigens or polypeptides from the pathogen.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i. e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or γ T cells; directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance of the mammary gland to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host and/or a quicker recovery time.

By "nucleic acid [IMMUNIZATION]" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the in vivo expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced ex vivo, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

The [TERM "TREATMENT" AS] used herein refers to either (1) the prevention of infection or reinfection (prophylaxis), or (2) the reduction or elimination of symptoms of the disease of interest (therapy).

By "mastitis" is meant an inflammation of the mammary gland in mammals, including in cows, ewes, goats, sows, mares, and the like, caused by the presence [OF S.] [UBERIS.] The infection manifests itself by the infiltration of phagocytic cells in the gland.

Generally, 4 clinical types of mastitis are recognized: (1) peracute, associated with swelling, heat, pain, and abnormal secretion in the gland and accompanied by fever and other signs of systemic disturbance, such as marked depression, rapid weak pulse, sunken eyes, weakness and complete anorexia; (2) acute, with changes in the gland similar to those above but where fever, anorexia and depression are slight to moderate; (3) subacute, where no systemic changes are displayed and the changes in the gland and its secretion are less marked; and (4) [SUBCLINICAL,] where the inflammatory reaction is detectable only by standard tests for mastitis.

Standard tests for the detection of mastitis include but are not limited to, the California Mastitis Test, the Wisconsin Mastitis Test, the [NAGASE] test, the electronic cell count and somatic cell counts used to detect a persistently high white blood cell content in milk. In general, a somatic cell count of about 300,000 to about

12

500,000 cells per ml or higher, in milk will indicate the presence of infection. Thus, a vaccine is considered effective in the treatment and/or prevention of mastitis when, for example, the somatic cell count in milk is retained below about 500,000 cells per ml. For a discussion of mastitis and the diagnosis thereof, see, e. g., [THE MERCK VETERINARY MANUAL : A HANDBOOK OF DIAGNOSIS,] Therapy, [AND DISEASE PREVENTION AND CONTROL FOR THE VETERINARIAN, MERCK] and Co., Rahway, New Jersey, [1991.]

By the [TERMS"VERTEBRATE,""SUBJECT,"AND"VERTEBRATE SUBJECT"ARE] meant any member of the subphylum [CHORDATA,] including, without limitation, mammals such as cattle, sheep, pigs, goats, horses, and humans; domestic animals such as dogs and cats; and birds, including domestic, wild and game birds such as cocks and hens including chickens, turkeys and other gallinaceous birds; and fish. The term does not denote a particular age. Thus, both adult and newborn animals, as well as fetuses, are intended to be covered. A"nucleic acid"molecule can include, but is not limited to, procaryotic sequences, eucaryotic [MRNA,] cDNA from eucaryotic [MRNA,] genomic DNA sequences from eucaryotic [(E.) g., mammalian] DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

[AN"ISOLATED"NUCLEIC] acid molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature; or a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith. The term"isolated" in the context of a polynucleotide intends that the polynucleotide is isolated from the chromosome with which it is normally associated, and is isolated from the complete genomic sequence in which it normally occurs.

"Purified [POLYNUCLEOTIDE"REFERS] to a polynucleotide of interest or fragment thereof which is essentially free, e. g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide (s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

A"coding sequence"or a"nucleotide sequence encoding"a particular protein, is a nucleotide sequence which is transcribed and translated into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory elements. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic [MRNA,] genomic DNA sequences from eucaryotic (e. g., mammalian) DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3'to the coding sequence. A"complementary" sequence is one in which the nitrogenous base at a given nucleotide position is the complement of the nitrogenous base appearing at the same position in the reference sequence. To illustrate, the complement of adenosine is tyrosine, and vice versa; similarly, cytosine is complementary to guanine, and vice versa; hence, the complement of the reference sequence 5'-ATGCTGA-3'would be 5'-TACGACT-3'.

A"wild-type"or"native"sequence, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e. g., the *S. dysgalactiae* Mig protein coding sequences depicted in Figures [IA-LD] (SEQ [ID] NO : 4).

"Recombinant"as used herein to describe a nucleic acid molecule means a polynucleotide [OF GENOMIC, CDNA,] semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The [TERM"RECOMBINANT"AS] used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide.

"Recombinant host cells,""host cells,""cells,""cell lines,""cell cultures,"and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used

as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation.

Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

"Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis, such as ALIGN, Dayhoff, M. O. in Atlas of Protein Sequence and Structure M. O.

[DAYHOFF ED.,] 5 Suppl. 3: [353-358,] National biomedical Research Foundation, Washington, DC, which adapts the local homology algorithm of Smith and Waterman (1981) Advances in Appl. Math. 2: 482-489 for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by [INTELLIGENETICS,] Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated [THE "MATCH" VALUE] reflects "sequence [IDENTITY." OTHER] suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters : genetic code = standard; filter = none; strand = both ; cutoff = 60; expect [-10] ; [-] [BLOSUM62] ; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: [http://www. \[NCBI.\] nhl. nih. \[GOV/BLAST/.\]](http://www.ncbi.nlm.nih.gov/BLAST/)

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease (s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e. g., Sambrook et al., supra ; DNA Cloning, supra ; Nucleic Acid Hybridization, supra.

By the term "degenerate variant" is intended a polynucleotide containing changes in the nucleic acid sequence thereof, that encodes a polypeptide having the same amino acid sequence as the

14

polypeptide encoded by the polynucleotide from which the degenerate variant is derived.

Techniques for determining amino acid [SEQUENCE "SIMILARITY" ARE] well known in the art. In [GENERAL, "SIMILARITY" MEANS] the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences.

Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the [MRNA] for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a bacterial gene, the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacteria. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e. g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring [MUTATIONAL] events do not give rise to a heterologous region of DNA, as used herein.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A vector is capable of transferring gene sequences to target cells (e. g., bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers, and liposomes).

Typically, the terms "vector [CONSTRUCT, "EXPRESSION] vector, "gene expression vector, "gene delivery vector, "gene transfer vector, "and "expression cassette" all refer to an assembly which is capable of directing the expression of a sequence or gene of interest.

Thus, the terms include cloning and expression vehicles, as well as viral vectors.

These assemblies include a promoter which is operably linked to the sequences or gene (s) of interest. Other control elements may be present as well. The expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA [(E.] g., a M13 origin of replication), a multiple cloning site, and [A "MAMMALIAN" ORIGIN] of replication (e. g., a SV40 or adenovirus origin of replication).

DNA "control elements" refers collectively to transcription promoters, transcription enhancer elements, transcription termination sequences, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), translation termination sequences, upstream regulatory domains, ribosome binding sites and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. See e. g., McCaughan et al. (1995) PNAS USA 92: 5431-5435; Kochetov et al (1998) FEBS Letts. 440: 351-355. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

"Operably [LINKED "REFERS] to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter and the coding sequence and the promoter can still be considered "operably [LINKED "TO] the coding sequence. Similarly, "control elements compatible with expression in a subject" are those which are capable of effecting the expression of the coding sequence in that subject.

A control element, such as a promoter, "directs the transcription" of

15

a coding sequence in a cell when RNA polymerase will bind the promoter and transcribe the coding sequence into [MRNA,] which is then translated into the polypeptide encoded by the coding sequence.

[A"HOST] cell" is a cell which has been transformed, or is capable of transformation, by an exogenous nucleic acid molecule. A cell has [BEEN"TRANSFORMED"BY] exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, external secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, organs, biopsies and also samples of [IN. VITRO] cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, e. g., recombinant cells, and cell components.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemilumescers, enzymes, enzyme substrates, enzyme factors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (e. g., biotin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. Particular examples of labels which may be used under the invention include fluorescein, rhodamine, dansyl, umbelliferone, Texas red, luminol, NADPH and [A- β -] galactosidase.

2. MODES OF CARRYING OUT THE INVENTION Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

Central to the present invention is the discovery that the Mig Fc binding protein isolated from *S. dysgalactiae* is capable of eliciting an immune response in a vertebrate subject. In particular, the gene for the Mig protein in *S. dysgalactiae* has been isolated, [SEQUENCED] and characterized, and the amino acid sequence encoded by the gene has been deduced therefrom. The complete DNA sequence for the [S.] *dysgalactiae* [MIG] gene (SEQ ID NO : 3) and the corresponding amino acid sequence (SEQ ID NO : 4) are shown in Figures [1A-] [1D.]

As described in the examples, the full-length [S.] *dysgalactiae* [MIG] gene, depicted at nucleotide positions 1-2,010 inclusive, of Figures [1A-1D,] encodes the full-length *S. dysgalactiae* Mig protein of 669 amino acids, which includes a first 24 amino acid transmembrane region (residues 15 and 39) and a second 18 amino acid transmembrane region of 18 amino acids (residues [646-664].)

Transmembrane regions were determined using the TMpred program, which predicts [MEMBRANE-SPANNING] regions and their orientation. The program uses an algorithm based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins, using a combination of several weight matrices for scoring. See Hofmann, [K.,] & Stoffel, W. (1993) Biol. Chem. 347: 166. *S. dysgalactiae* Mig has a predicted molecular weight of about 73 kDa. (calculated using the Peptide Sort program of the GCG Wisconsin Package, version, provided by the SeqWeb sequence analysis package, version 1.1 of the Canadian [BIOINFORMATICS] Resource). The full-length sequence includes a signal peptide.

Figure 2 plots the following structural analyses results for the Mig

protein of the present invention: Kyte-Doolittle hydrophathy, averaged over a window of 7; surface probability according to [EMINI] ; chain flexibility according to Karplus-Schulz ; antigenicity index according to Jameson-Wolf; secondary structure according to Garnier-Osguthorpe-Robson; secondary structure according to Chou-Fasman; and predicted glycosylation sites. Figure 3 plots secondary structure according to Chou-Fasman for the Mig protein of the present invention. One of skill in the art can readily use the information presented in Figures 2 and 3 to determine immunogenic regions in the protein for use in vaccine compositions.

Mig Fc receptor proteins, including without limitation the [S.] dysgalactiae Mig protein, immunogenic fragments thereof or chimeric proteins including the same, can be provided in subunit vaccine compositions. In addition to use in vaccine compositions, the proteins or antibodies thereto can be used as diagnostic reagents to detect the presence of infection in a vertebrate subject. Similarly, the genes encoding the proteins can be cloned and used to design probes to detect and isolate homologous genes in other bacterial strains.

For example, fragments comprising at least about 15-20 nucleotides, more preferably at least about 20-50 nucleotides, and most preferably about 60-100 nucleotides, or any integer between these values, will find use in these embodiments.

The vaccine compositions of the present invention can be used to treat or prevent a wide variety of bacterial infections in vertebrate subjects. For example, vaccine compositions including, without limitation, the Mig Fc receptor protein from [S.] dysgalactiae, can be used to treat streptococcal infections in vertebrate subjects that are caused by this or other species. In particular, *S. uberis*, *S. agalactiae*, and [S.] dysgalactiae are common bacterial pathogens associated with mastitis in bovine, equine, ovine and goat species.

Additionally, group B streptococci, such as [S.] agalactiae, are known to cause numerous other infections in vertebrates, including septicemia, meningitis, [BACTEREMIA,] impetigo, arthritis, urinary tract infections, abscesses, spontaneous abortion etc. Hence, vaccine compositions containing the Mig Fc receptor protein will find use in treating and/or preventing a wide variety of streptococcal infections.

Similarly, Fc binding proteins derived from other bacterial genera such as *Staphylococcus* will find use for treating bacterial infections caused by species belonging to those genera. Thus, it is readily apparent that Fc binding proteins from a variety of bacterial species can be used to treat and/or prevent a wide variety of bacterial infections in numerous animal species.

The streptococcal Fc binding proteins of the present invention, including without limitation the Mig Fc binding protein, can be used in vaccine compositions either alone or in combination with other bacterial, [FUNGAL,] viral or protozoal antigens. These antigens can be provided separately or even as fusion proteins comprising one or more epitopes of an Fc binding protein fused to one or more of these antigens. For example, other immunogenic proteins from *S. uberis*, such as the CAMP factor, hyaluronic acid capsule, hyaluronidase, R-like protein and plasminogen activator, can be administered with the Mig protein.

Additionally, immunogenic proteins from other organisms involved in mastitis, such as from the genera *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Nocardia*, *Clostridium*, *Mycobacterium*, [MYCOPLASMA] *Pasteurella*, *Prototheca*, other streptococci, [COLIFORM] bacteria, as well as yeast, can be administered along with the Fc binding proteins described herein to provide a broad spectrum of protection. Thus, for example, immunogenic proteins from *Staphylococcus* [AUF*EUS, STR. AGALACTIAE, ST. DYSGALACTIAE, STR. ZOOEPIDEMICUS,] [CORYNEBACTERIUM PYOGENES, PSEUDOMONAS AERUGINOSA, NOCARDIA ASTEROIDES, CLOSTRIDIUM] [PERFRINGENS, ESCHERICHIA COLI, ENTEROBACTER AEROGENES] and *Klebsiella* spp. can be provided along with the Fc binding proteins of the present invention.

Additionally, Fc proteins from different streptococcal species may be used together in the vaccine compositions of the present invention. In this embodiment, the multiple Fc proteins may be provided either as fusion proteins or as discrete antigens in the same or different vaccine compositions.

Production [OF Fc BINDING] Proteins The above-described Fc binding proteins and active fragments, analogs and chimeric proteins derived from the same, can be produced by variety of methods. Specifically,

12

Fc binding proteins can be isolated directly from bacteria which express the same. This is generally accomplished by first preparing a crude extract which lacks cellular components and several extraneous proteins. The desired proteins can then be further purified from the cell lysate fraction by, e. g., column chromatography, [HPLC,] [IMMUNOADSORBENT] techniques or other conventional methods well known in the art. More particularly, techniques for isolating Fc binding proteins have been described.

For example, the methods of Reis, et al. have been used to isolate a functionally homogeneous Fc receptor having the properties of a type-III receptor (Reis et al., (1984) [J.] Immunol. 132: 3091).

Alternatively, the proteins can be [RECOMBINANTLY] produced as described herein. As explained above, these recombinant products can take the form of partial protein sequences, full-length sequences, precursor forms that include signal sequences, mature forms without signals, or even fusion proteins (e. g., with an appropriate leader for the recombinant host, or with another subunit antigen sequence for Streptococcus or another pathogen).

In one embodiment of the present invention, the [S.] dysgalactiae Mig protein is purified from a cell lysate fraction using affinity chromatography after recombinantly producing the protein. See Example [1A-D,] infra.

Gene libraries can be constructed and the resulting clones used to transform an appropriate host cell. Colonies can be pooled and screened for clones having Fc receptor binding activity, i. e., for clones capable of binding IgG.

Alternatively, once the amino acid sequences are [DETERMINED,] [OLIGONUCLEOTIDE] probes which contain the codons for a portion of the determined amino acid sequences can be prepared and used to screen genomic or cDNA libraries for genes encoding the subject proteins. The basic strategies for preparing [OLIGONUCLEOTIDE] probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e. g., DNA Cloning : Vol. 1I, supra ; Nucleic Acid Hybridization, supra ; [OLIGONUCLEOTIDE] Synthesis, supra; Sambrook et al., supra. Once a clone from the screened library has been identified by positive hybridization, it can be confirmed by restriction enzyme analysis and DNA sequencing that the particular library insert contains a Fc binding protein gene or a homolog thereof. The genes can then be further isolated using standard techniques and, if desired, PCR approaches or restriction enzymes employed to delete portions of the full-length sequence.

Similarly, genes can be isolated directly from bacteria using known techniques, such as phenol extraction and the sequence further manipulated to produce any desired alterations.

See, e. g., Sambrook et al., supra, for a description of techniques used to obtain and isolate DNA.

Alternatively, DNA sequences encoding the proteins of interest can be prepared synthetically rather than cloned. The DNA sequences can be designed with the appropriate codons for the particular amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e. g., Edge (1981) Nature 292: 756; Nambair et al. (1984) Science 223: 1299; Jay et al. (1984) J. Biol. [CHEM.] 259: 6311.

Once coding sequences for the desired proteins have been prepared or isolated, they can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage [X] (E. [COLI],) pBR322 (E. [COLI],) pACYC177 (E. coli), pKT230 (gram-negative bacteria), [PGVL 106] (gram-negative bacteria), [PLAFRL] (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV14 (E. coli and [BACILLUS SUBTILIS]), PBD9 ([BACILLUS]), pIJ61 [(STREPTOMYCES),] pUC6 [(STREPTOMYCES),] Yip5 [(NACCHAROMYCES),] [YCPL9 (SACCHAROMYCES)] and bovine papilloma virus (mammalian cells).

See, Sambrook et al., supra ; DNA Cloning, supra ; B. Perbal, supra.

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator

18

(collectively referred to herein [AS"CONTROL"] elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. If a signal sequence is included, it can either be the native, homologous sequence, or a heterologous sequence. For example, the signal sequence for the *S. dysgalactiae* Mig protein (amino acid residue 1 to 39, inclusive) can be used for secretion thereof, as can a number of other signal sequences, well known in the art. Leader sequences can be removed by the host in post-translational processing. See, e. g., U. S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above.

Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation; i. e., to maintain the proper reading frame. It may also be desirable to produce mutants or analogs of the Fc binding protein. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, [AND/OR] by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are described in, e. g., Sambrook et al., supra; DNA Cloning, supra; Nucleic Acid Hybridization, supra.

The expression vector is then used to transform an appropriate host cell. A number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human [HEPATOCELLULAR] carcinoma cells (e. g., Hep G2), Madin-Darby bovine kidney [{"MDBK"}] cells, as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus* [SUBTILIS, AND STREPTOCOCCUS SPP.,] will find use with the present expression constructs. Yeast hosts useful in the present invention include inter alia, *Saccharomyces cerevisiae*, *Candida* [

 ALBICANS, CAFZDIDA MALTOSA, HANSENULA POLYMOPHA, KLUYVEROMYCES FYAGILIS, KLUYVEROMYCES] *lactis*, *Pichia* [GUILLERIMONDII, PICHIA PASTORIS, SCHIZOSACCHAROMYCES POMBE AND YARROWIA] *lipolytica*. Insect cells for use with baculovirus expression vectors include, [INTER ALIA, AEDES] *aegypti*, [AUTOGRAPHIA CALIFORNICA, BOMBYX MORI, DROSOPHILA MELANOGASTER, SPODOPTERA] *frugiperda*, and *Trichoplusia ni*.

Depending on the expression system and host selected, the proteins of the present invention are produced by culturing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into the growth media, the protein can be purified directly from the media. If the protein is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The proteins of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis, using known amino acid sequences or amino acid sequences derived from the DNA sequence of the genes of interest. Such methods are known to those skilled in the art. See, e. g., J. M. Stewart and J. D. Young, Solid Phase Peptide [SYNTHESIS, 2ND] Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R.

B. [MERRIFIELD,] The Peptides : Analysis, Synthesis, Biology, editors E. Gross and J.

[MEIENHOFER,] Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. [BODANSKY,]

14

[PRINCIPLES OF PEPTIDE SYNTHESIS,] Springer-Verlag, Berlin [(1984)] and E. Gross and J. Meienhofer, Eds., The Peptides : Analysis, Synthesis, Biology, supra, Vol. 1, for classical solution synthesis. Chemical synthesis of peptides may be preferable if a small fragment of the antigen in question is capable of raising an immunological response in the subject of interest.

The Fc binding proteins of the present invention, or their fragments, can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal, (e. g., mouse, rabbit, goat, horse, etc.) is immunized with an antigen of the present invention, or its fragment, or a mutated antigen. Serum from the immunized animal is collected and treated according to known procedures. See, e. g., Jurgens et al. (1985) J Chrom. 348: 363-370. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by [IMMUNOAFFINITY] chromatography, using known procedures.

Monoclonal antibodies to the Mig protein and to the fragments thereof, can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by using hybridoma technology is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e. g., M. Schreier et al., Hybridoma Techniques (1980); Hammerling et al., Monoclonal Antibodies [AND T-CELL HYBRIDOMAS (1981)] ; [KENNETT] et al., Monoclonal [ANTIBODIES (1980)] ; see also U. S.

Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,452,570; 4,466,917; 4,472,500, 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against the Mig protein, or fragments thereof, can be screened for various properties; i. e., for isotype, epitope, affinity, etc. Monoclonal antibodies are useful in purification, using immunoaffinity techniques, of the individual antigens which they are directed against. Both polyclonal and monoclonal antibodies can also be used for passive immunization or can be combined with subunit vaccine preparations to enhance the immune response. Polyclonal and monoclonal antibodies are also useful for diagnostic purposes.

Vaccine Formulations and Administration The Fc binding proteins of the present invention can be formulated into vaccine compositions, either alone or in combination with other antigens, for use in immunizing subjects as described below. Methods of preparing such formulations are described in, e. g., [RENNINGTON&APOS;S PHARMACEUTICAL SCIENCES, MAELC] Publishing Company, Easton, Pennsylvania, 18 Edition, 1990. Typically, the vaccines of the present invention are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in or suspension in liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active immunogenic ingredient is generally mixed with a compatible pharmaceutical vehicle, such as, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents.

Adjuvants which enhance the effectiveness of the vaccine may also be added to the formulation. Adjuvants may include for example, [MURAMYL] dipeptides, avridine, aluminum hydroxide, dimethyldioctadecyl ammonium bromide (DDA), oils, oil-in-water emulsions, saponins, [CYTOLDNES,] and other substances known in the art.

The Mig protein may be linked to a carrier in order to increase the immunogenicity thereof. Suitable carriers include large, slowly metabolized [MACROMOLECULES] such as proteins, including serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well [KNOWN] to those skilled in the art; polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids such as polyglutamic acid, polylysine, and the like ; amino acid copolymers; and inactive virus particles.

The Fc binding proteins of the present invention may be used in their native form or their functional group content may be modified by, for example, [SUCCINYLTATION] of lysine residues or reaction with Cys-thiolactone. A sulfhydryl group may also be incorporated into the carrier (or antigen) by, for example, reaction of amino functions with 2-iminothiolane or the [N-HYDROXSUCCINIMIDE] ester

of [3- (4-DITHIOPYRIDYL) propionate. Suitable carriers may also be modified to incorporate spacer arms (such as [HEXAMETHYLENE] diamine or other bifunctional molecules of similar size) for attachment of peptides.

Other suitable carriers for the Fc binding proteins of the present invention include VP6 polypeptides of rotaviruses, or functional fragments thereof, as disclosed in U. S. Patent No. 5,071,651. Also useful is a fusion product of a viral protein and the subject immunogens made by methods disclosed in U. S. Patent No. 4,722,840. Still other suitable carriers include cells, such as lymphocytes, since presentation in this form mimics the natural mode of presentation in the subject, which gives rise to the immunized state. Alternatively, the proteins of the present invention may be coupled to erythrocytes, preferably the subject's own erythrocytes. Methods of coupling peptides to proteins or cells are known to those of skill in the art.

Furthermore, the Fc binding proteins (or complexes thereof) may be formulated into vaccine compositions in either neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, [TRIMETHYLAMINE,] 2-ethylamino ethanol, histidine, procaine, and the like.

Vaccine formulations will contain a "therapeutically effective amount" of the active ingredient, that is, an amount capable of eliciting an immune response in a subject to which the composition is administered. In the treatment and prevention of mastitis, for example, a "therapeutically effective amount" would preferably be an amount that enhances resistance of the mammary gland to new infection and/or reduces the clinical severity of the disease. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time [AND/OR] a lowered somatic cell count in milk from the infected quarter. For example, the ability of the composition to retain or bring the somatic cell count (SCC) in milk below about 500,000 cells per ml, the threshold value set by the International Dairy Federation, above which, animals are considered to have clinical mastitis, will be indicative of a therapeutic effect. The exact amount is readily determined by one skilled in the art using standard tests.

The Fc binding protein concentration will typically range from about [1%] to about 95% (w/w) of the composition, or even higher or lower if appropriate. With the present vaccine formulations, 5 to [500 G] of active ingredient per ml of injected solution should be adequate to raise an immunological response when a dose of 1 to 3 ml per animal is administered.

To immunize a subject, the vaccine is generally administered parenterally, usually by intramuscular injection. Other modes of administration, however, such as subcutaneous, intraperitoneal and intravenous injection, are also acceptable. The quantity to be administered depends on the animal to be treated, the capacity of the animal's immune system to synthesize antibodies, and the degree of protection desired. Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves. The subject is immunized by administration of the vaccine in at least one dose, and preferably two doses. Moreover, the animal may be administered as many doses as is required to maintain a state of immunity to infection.

Additional vaccine formulations which are suitable for other modes of administration include suppositories and, in some cases, aerosol, intranasal, oral formulations, and sustained release formulations. For suppositories, the vehicle composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about [1%] to about 2%. Oral vehicles include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release

formulations, or powders, and contain from about 10% to about 95% of the active ingredient, preferably about 25% to about 70%. Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and [BENZALKONIUM] chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the protein into carriers or vehicles such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and [HYTREL] copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make [RESORBABLE] sutures. The Fc binding proteins can also be delivered using implanted mini-pumps, well known in the art.

The Fc binding proteins of the instant invention can also be administered via a carrier virus which expresses the same. Carrier viruses which will find use with the instant invention include but are not limited to the vaccinia and other pox viruses, adenovirus, and herpes virus. By way of example, vaccinia virus recombinants expressing the novel proteins can be constructed as follows. The DNA encoding the particular protein is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the instant protein into the viral genome. The resulting [TKRECOMBINANT] can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

An alternative route of administration involves gene therapy or nucleic acid immunization. Thus, nucleotide sequences (and accompanying regulatory elements) encoding the subject Fc binding proteins can be administered directly to a subject for in vivo translation thereof. Alternatively, gene transfer can be accomplished by transfecting the subject's cells or tissues ex vivo and reintroducing the transformed material into the host.

DNA can be directly introduced into the host organism, i. e., by injection (see International Publication No. WO/90/11092; and Wolf et al. (1990) [SCIENCE 247-.] 1465-1468). Liposome-mediated gene transfer can also be accomplished using known methods. See, e. g., Hazinslci et al. [(1991)] Am. J. [RESPIR. CELL MOL. BIOL.] 4: 206-209; Brigham et al. [(1989)] Am. J. Med. Sci. 298: 278-281; Canonico et al. (1991) Clin. Res. 39: 219A; [AND NABEL] et al. (1990) Science 249: [1285-1288.] Targeting agents, such as antibodies directed against surface antigens expressed on specific cell types, can be covalently conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells susceptible to infection.

Deposits of Strains Useful in Practicing the Invention A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 10801 University Boulevard, Manassas. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for a period of [THIRTY] (30) years from the date of deposit. The organisms will be made available by the ATCC under the terms of the Budapest Treaty, which assures permanent and unrestricted availability of the progeny to one determined by the U. S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U. S. C. §122 and the Commissioner's rules pursuant thereto (including 37 C. F. R. [§1.] 12 with particular reference to 886 OG 638). Upon the granting of a patent, all restrictions on the availability to the public of the deposited cultures will be irrevocably removed.

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35 U. S. C. § 112. The nucleic acid sequences of these genes, as well as the amino acid sequences of the molecules encoded thereby, are incorporated herein by reference and are controlling in

the event of any conflict with the description herein.

Bacterial Plasmid Gene Deposit Date ATCC No.

Strain DH5a pAA505Mig [S. DYSGALACTIAE] May 31,2000 PTA-1977 mig gene EXPERIMENTAL Below are examples of specific embodiments for carrying out the present invention.

The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e. g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Materials and Methods Enzymes were purchased from commercial sources, and used according to the manufacturers' directions.

In the isolation of DNA fragments, except where noted, all DNA manipulations were done according to standard procedures. See, Sambrook et al., supra. Restriction enzymes, [T4 DNA] ligase, E. coli, DNA polymerase [I,] Klenow fragment, and other biological reagents can be purchased from commercial suppliers and used according to the manufacturers' directions. Double stranded DNA fragments were separated on agarose gels.

EXAMPLE 1 Preparation, Amplification, [SEQUENCING,] Expression, Purification and Characterization of the [S. DVSGALACTIAE MIG] Fc Receptor Protein [A. PREPARATION OF S. DVSGALACTIAE CHROMOSOMAL] DNA A clinical [S.] dysgalactiae isolate from a case of bovine mastitis (ATCC Accession No. 43078) was obtained from the American Type Culture Collection (10801 University Boulevard, Manassas, VA [20110-2209],) and was used as a source of DNA. The organism was routinely grown on TSA sheep blood agar plates (PML Microbiologicals, Mississauga, Ontario) at 37° C for 18 hours, or in Todd-Hewitt broth (Oxoid Ltd., Hampshire, England) supplemented with 0.3% yeast extract (Sigma, St. Louis, Missouri) [(THB-YE)] at 37° C, 5% [CL2.]

Chromosomal DNA was prepared from S. dysgalactiae grown in 100 ml of THB-YE supplemented with 20 mM glycine for approximately 6 hours, until an [AGOO] of [0. 8] to 1.0 was reached. Cells were harvested and re-suspended in 50 mM EDTA, 50 mM [TRIS-HCL,] 0.5% TWEEN-20 (Sigma, St. Louis, MO) and supplemented with RNase A (200 mg/ml), proteinase K (20 mg/ml), lysozyme (100 [MG/ML]) and mutanolysin (100 mg/ml). (Sigma, St.

Louis, Missouri). Following bacterial lysis for 30 minutes at 37° C with vigorous shaking, guanidine hydrochloride and TWEEN-20, pH 5.5, were mixed with the lysate to give a final concentration of 0.8 M and 5%, respectively. This mixture was incubated at [50° C] for 30 minutes. The chromosomal DNA was then purified using a Qiagen genomic-tip 100g [(QIAGEN,] Germany) and precipitated using 0.7 volumes of isopropanol. The resulting pellet was washed in 70% ethanol and re-suspended in 0.5 ml 10 mM [TRIS-HCL,] pH 8.8.

B. Amplification and Cloning of the S. [DVSGALACTIAE MIG GENE] The Mig gene was amplified by PCR (See Mullis et al., U. S. Patent No. [4,] 683,195; Mullis, U. S. Patent No. 4,683,202;) using the forward primer [MIGL] (SEQ ID NO : [1,] shown in Table [1]) and the reverse primer miglr (SEQ ID NO : 2, shown in Table 1). In the sequences depicted in Table 1, underlining denotes nucleotides added to the original sequence, and holding indicates the location of restriction endonuclease recognition sites. TABLE [1] Tabulation of Sequences

SEQUENCE SEQUENCE ID NO: NAME NUCLEOTIDE/AMINO ACID SEQUENCE Primer rnigl 5'-G CGG CCA TGG TAG AAA ATA CTA TAA CTG-3' 2 Primer rniglR 5'-ACG CCC GGG TTA GTC TTC TTT ACG TTT-3' 3Streptococcus dysgalactiae strainSDG8 fig genre (see Figure 1) 4Streptococcus dysgalactiae strain SDG8 Mig protein 5 Primer mig-3 5'-GTT GGC CTA GAT ATC ACA GAA TTA CAA-3' 6 Primer mig-4 5'-AAA GCA CCC GGG CCA GCC ATT ACT G-3' 7 Primer nzig-6 5'-AGG TGC TTC CCA TGG AAC TGC CTG AAC T-3' 8 Primer mig-7 5'-GGC GAG AGT CTA GAA ACT AAA GCG AAA AAC-3' 9 Primer mig-8 5'-GCA ATC ACC AGG ATC CTC AGT AAC CAT TTC-3' 10 Primer inig-9 5'-CAG GCA GTT CAT ATG GAA GCA CCT ACA GT-3' 11 Primer nzig-10 5'-TCC CGG AGT AGC ATT GTC AGT C-3' 12 Primer mig-11 5'-GCA GCG GTC CAT ATG CCT GTT GGC CTA GAT-3' 13 Primer mig-12 5'-GCC TGA ACT GGA TCC CTC AAC TGA TCT G-3' 14 Primer Mg-7 3 5'-TTC CGT TGG ATC CTG CAA CTC CAA TTG-3' 15 Primer nag-14 5'-TAA GTC AAA AGC TTT GAC AAT TAG TCT T-3' PCR was carried out using Vent DNA polymerase (New England Biolabs, Mississauga, Ontario, Canada). 0.711g of S. dysgalactiae genomic DNA, [1, UM] each of the [MH&APOS;G7] (SEQ ID NO : 1) [77SIGL R (SEQ] ID NO : 2) primers (see above), 200 aM each [OF DATP,] dCTP, dGTP and [DTTP,] [3MM MGSO4, 1X THERMOPOL] PCR

buffer (New England Biolabs), and 2 units of Vent DNA Polymerase were combined. The reaction mix was then incubated for 3 cycles of 1 minute at [94°C,] 3 minutes at [50°C,] and 1 minute, 10 seconds at [72°C,] followed by 27 cycles of 15 seconds [AT 95°C,] 30 seconds [AT 55°C,] 1 minute [AT 72°C,] followed by a single cycle of 5 minutes at [72°C.]

The mig PCR product obtained above and the expression vector pAA505 (VIDO, [SASKATOON,] Saskatchewan, Canada) were digested with the restriction enzymes [NCOI AND] [SIIIAL] (Amersham Pharmacia, Quebec, Canada) according to the manufacturer's instructions, and the [MIG] sequence was ligated into the same sites of the vector.

This construct was used to transform E. coli DH5 a (Life Technologies, Gaithersburg, MD). The transformed E. coli DH5 a cells bearing the pAA505-mig vector construct were designated [E.] coli DH5a [PAA505MIG.]

[C. NUCLEOTIDE SEQUENCE OF THE S. DYSGALACTIAE MIG GENE] and the Corresponding Deduced Amino Acid Sequence The nucleotide sequence in both orientations of the mig gene was determined on an ABI 373 DNA automatic sequencer (Applied [BIOSYSTEMS]) at the Plant Biotechnology Institute (PBI, Saskatoon, Saskatchewan, Canada) by using the multiple primers shown in Table 1 (Primers [MIG-2] [THROUGH MIG-14].)

Figures [LA-LD DEPICT] the nucleotide coding sequence and the amino acid sequence, respectively for the [S.] dysgalactiae Mig protein (SEQ ID NO : 3 and SEQ ID NO : 4, respectively).

These sequences were then compared against known sequences via BLAST analysis.

The search parameters used to analyze the nucleic acid sequence were as follows: Database: nt; Number of letters in database:

1,961,177,913; Number of sequences in database: 614,801; Matrix: blastn matrix: [1-3] ; Gap Penalties: Existence : 5, [EXTENSION : 2.] The results obtained showed that the S. dysgalactiae SD8 mig gene depicted in Figures [1A-ID] is homologous to several known nucleotide sequences, e. g., there is 98% homology with the mig gene for S. dysgalactiae SC1 (Emb Accession No. Z29666.1 SDMIGSUP).

The search parameters used to analyze the amino acid sequence were as follows: Database: [NR ; NUMBER] of letters in database:

157,988,256; Number of Sequences in database: 503,479; Matrix: BLOSUM62 ; Gap Penalties: Existence : [11,] Extension: 1. The results obtained showed that the S. [DYSGALACTIAE] SD8 mig amino acid sequence depicted in Figures 1A-1D is up to 89% homologous to several known amino acid sequences.

D. Expression and Purification of the Recombinant S. [DYSGALACTIAE MIG] Protein E. coli containing the construct prepared in Example 1B, supra, was grown in LB broth containing 100 [µg/mL] ampicillin to an A₆₀₀ of approximately 0.5. Expression of the Mig protein was then induced by the addition of 1 mM [ISOPROPYL-β,]

D-thiogalactoside (IPTG) (Sigma, St. Louis, MO). Following three hours incubation at [37°C,] cells were harvested, washed in column buffer [(50mM) sodium phosphate pH 8.0, 0.2 M [NaCl]] and lysed by sonication.

Approximately 40% of the recombinant protein was in the soluble fraction of the cell sonicate with a yield of approximately 50 mg of the recombinant protein per litre of culture volume, as determined using a DC Protein Assay kit (BioRad Laboratories, Mississauga, Ontario, Canada) with bovine serum albumin (Pierce, [ROCKFORD,] Illinois) as a standard.

The recombinant Mig protein was purified by affinity chromatography using an agarose-IgG matrix, based upon the protein's ability to bind the Fc portion of the IgG molecule. The cell lysate was cleared by centrifugation and the soluble fraction was applied to a [BLIGG] agarose column (Sigma, St. Louis, Missouri). The column was washed with 10 column volumes of column buffer (50 mM sodium phosphate pH 8.0, 0.3 M [NaCl],) and the protein eluted with column buffer plus 0.1 M glycine, pH 2.5, yielding a homogeneous protein fraction with a Mig concentration of approximately 10-15 mg/ml. The eluate was dialyzed against 2,000 volumes [OF PBSA] and stored at -20 °C.

E. Characterization of the Recombinant Protein Analysis of the purified protein by SDS-PAGE demonstrated 60% purity.

EXAMPLE 2 Immunization with Mig and experimental infection of cattle Vaccines were formulated to contain 50 mg/ml of affinity purified recombinant Mig or GapC in the oil-based adjuvant VSA3 (VIDO, [SASKATOON, SASKATCHEWAN,] Canada). VSA3 is a combination [OF EMULSIGEN PLUS™] (MVP Laboratories, Ralston, Nebraska) and Dimethyldioctadecyl ammonium bromide [(KODAK,] Rochester, New York)...

24

24 non-lactating Holsteins with no history [OF S. DYSGALACTIAE] infection were obtained from various farms in Saskatchewan, Canada. One week prior to vaccination, all animals were treated with [CEPHA-DRYTM] (300 mg per quarter ; Ayerst Laboratories, Montreal, Canada), in order to clear any infection of the udders prior to the vaccination step.

Three groups of eight animals were immunized [SUBCUTANEOUSLY] with two doses of vaccines containing Mig, GapC (a plasmin binding protein isolated from streptococcus bacteria that was evaluated simultaneously), or a placebo with a three-week interval between immunizations. Two weeks following the second immunization, animals were exposed to 650 colony forming units of S. dysgalactiae delivered into three quarters with an udder infusion cannula. The fourth quarter on each animal served as an un-infective control. All animals were examined daily for clinical signs of disease and samples from all udder quarters were collected on each day. Samples were observed for consistency and somatic cell counts as well as bacterial numbers were determined.

EXAMPLE 3 Bacterial Colonization Bacteria were enumerated by spreading serial dilutions [(10⁶ TO 10⁻³)] directly onto TSA sheep blood agar plates followed by overnight incubation at [37°C,] 5% CO₂. Colonization is defined as >500 [CFU/ML] of the challenge organism recovered.

To confirm that the bacteria recovered from milk secretions were S. dysgalactiae, selected colonies recovered from each animal were tested using an API strep-20 test [(BIOMERIEUX) SA, Hazelwood, Missouri] according to the manufacturer's instructions. This test is a standardized method which combines 20 biochemical assays for [ENZYMATIC] activity and sugar fermentation, the results of which yield an analytical profile. The profile permits identification of the particular streptococcal species present by either referring to an analytical profile index or using identification software. Following challenge with S. dysgalactiae, animals from all groups were shown to be colonized by S. dysgalactiae (Figure 4). The Mig-immunized animals showed a reduction in the number of infected quarters on days three and four post-challenge.

EXAMPLE 4 Measuring Inflammatory Response Inflammatory response was measured as a function of somatic cell count (i. e., lymphocytes, neutrophils, and monocytes). Somatic cell counts were measured in a Coulter counter using standard techniques, as recommended by Agriculture and Agri-Food Canada Pamphlet [IDF50B] (1985) [MILK AND MILKPRODUCTS--METHODS OF SAMPLING.] Samples were always read within 48 hours of collection and fixation.

The number of somatic cells present in the gland was determined at days 1 through 7 post-challenge. Numbers from the unchallenged quarter remained constant throughout the trial, while on day [1,] the Mig-immunized group was lower than the placebo-immunized group (Figure 5). The individual data from day 1 is shown in Figure 6. Thus, the cloning, expression and characterization of the [S.] dysgalactiae Mig protein are disclosed, as are methods of using the same.

Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

Description Claims

SDOCL LA="EN">

CLAIMS What is claimed is: 1. A vaccine composition comprising a pharmaceutically acceptable vehicle and an Fc receptor protein.

2. The vaccine composition of claim 1, wherein said Fc receptor protein is selected from the group consisting [OF :] (a) a Streptococcus dysgalactiae Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ [ID] NO : 4); (b) an Fc receptor protein having at least about 70% sequence identity to (a); and [(C)] immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

3. The vaccine composition of claim 2, wherein said Mig protein comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures [1A-1D] (SEQ ID NO : 4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

4. The vaccine composition of claim 3, wherein said Mig protein

comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO : 4).

5. The vaccine composition of claim 1, further comprising an adjuvant.

6. A method of producing a vaccine composition comprising the steps of (a) providing an Fc receptor protein or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids, and (b) combining said protein with a pharmaceutically acceptable vehicle.

7. Use of an Fc receptor protein for the manufacture of a vaccine composition useful for treating or preventing a bacterial infection in a vertebrate subject.

8. The use according to claim 7, wherein the Fc receptor protein is selected from the group consisting [OF :] (a) a Streptococcus dysgalactiae Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO : 4); (b) an Fc receptor protein having at least about 70% sequence identity to (a); and (c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

9. The use according to claim 8, wherein said Mig protein comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO : 4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

10. The use according to claim 9, wherein said Mig protein comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures [1A-1D] (SEQ ID NO : 4).

[11.] The use according to any of claims 7-10, wherein said bacterial infection is a streptococcal infection.

12. The use according to claim 11, wherein said bacterial infection causes mastitis.

13. Use of a polynucleotide comprising a coding sequence for an Fc receptor protein in the manufacture of a medicament useful for treating or preventing a bacterial infection in a vertebrate subject.

14. The use according to claim 13, wherein the Fc receptor protein is selected from the group consisting [OF :] (a) a Streptococcus dysgalactiae Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures [1A-1D] (SEQ ID NO : 4); (b) an Fc receptor protein having at least about 70% sequence identity to (a); and (c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

15. The use according to claim 14, wherein said Mig protein comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures [1A-1D] (SEQ ID NO : 4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

16. The use according to claim 15, wherein said Mig protein comprises the amino acid sequence of the Streptococcus dysgalactiae Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO : 4).

17. The use according to any of claims 13-16, wherein said bacterial infection is a streptococcal infection.

18. The use according to claim 17, wherein said bacterial infection causes mastitis.

19. A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to any of claims 1-5.

20. The method of claim 19 wherein said bacterial infection is a streptococcal infection.

21. The method of claim 20 wherein said bacterial infection causes mastitis.

22. A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a polynucleotide, said polynucleotide comprising a coding sequence for an Fc receptor protein.

23. The method of claim 22, wherein the Fc receptor protein is selected from the group consisting [OF :] (a) a Streptococcus dysgalactiae Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO : 4); (b) an Fc receptor protein having at least about 70% sequence identity to (a); and (c) immunogenic fragments of (a) and

(b), said fragments comprising at least about 5 amino acids.

24. The method of claim 23, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures [IA-ID] (SEQ ID NO : 4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

25. The method of claim 24, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures [IA-ID] (SEQ ID NO : 4).

26. The method of any of claims 22-25, wherein said bacterial infection is a streptococcal infection.

27. The method of claim 26, wherein said bacterial infection causes mastitis.

Description Claims

27

PCT

REQUEST

The undersigned requests that the present
International application be processed
according to the Patent Cooperation Treaty

or receiving Office use only

PCT/CA 01/00837

International Application No.

11 JUNE 2001 (11 06 01)

International Filing Date

PCT/CA

Name of receiving Office and PCT International Application

Applicant's or agent's file reference
(if desired) (12 characters maximum) 08-891815W0

Box No. I TITLE OF INVENTION

Immunization Of Dairy Cattle With Mlg Protein

Box No. II APPLICANT

☐ This person is also inventor

Name and address: (Family name followed by given name for a legal entity, full official designation.
The address must include postal code and name of country. The country of the address indicated in this
Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

University of Saskatchewan
120 Veterinary Road
Saskatoon Saskatchewan
CANADA S7N 5E3

Telephone No.

306 - 996-7465

Facsimile No.

306 - 996-7478

Teleprinter No.

Applicant's registration No. with the Office

State (that is, country) of nationality

CA

State (that is, country) of residence:

CA

This person is applicant
for the purposes of:☒ all designated
States☐ all designated States except
the United States of America☐ the United States
of America only☐ the States indicated in
the Supplemental Sheet

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name for a legal entity, full official designation.
The address must include postal code and name of country. The country of the address indicated in this
Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

POTTER Andrew A
521 Dalhousie Crescent
Saskatoon Saskatchewan
CANADA S7H 3S5

This person is:

☐ applicant only☐ applicant and inventor☒ inventor only (If this check-box
is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality

State (that is, country) of residence:

This person is applicant
for the purposes of:☐ all designated
States☐ all designated States except
the United States of America☐ the United States
of America only☐ the States indicated in
the Supplemental Sheet☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE- OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf
of the applicant(s) before the competent International Authorities as:

☒ agent☐ common
representative

Name and address: (Family name followed by given name for a legal entity, full official designation.
The address must include postal code and name of country.)

ERRATT Judy A SECHLEY Konrad A
D'Iorio Helene Dudley Bruce
Govlings Lafleur Henderson LLP
160 Elgin Street Suite 2600
Ottawa Ontario
CANADA K1P 1C3

Telephone No.

613 - 233-1781

Facsimile No.

613 - 563-9869

Teleprinter No.

Agent's registration No. with the Office

☐ Address for correspondence: Mark this check-box where an agent or common representative is/has been appointed and the
space above is used instead to indicate a special address to which correspondence should be sent.

HO/CA

F T / CA 01/00837

28

2

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
If none of the following sub-boxes is used, this sheet should not be included in the request.	
Name and address: (Family name followed by given name; for a legal entity full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) BOLTON Alexandra J 115-1540 29th Street N W Calgary Alberta CANADA T2N 4M1	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) SONG Xin Ming #8 1506 Main Street Saskatoon Saskatchewan CANADA S7H 0L7	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

PCT/CA 01/00837

29

3

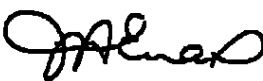
Box No. V DESIGNATION OF STATES		Mark the applicable check-boxes below. At least one must be marked.
The following designations are hereby made under Rule 4.9(a)		
Regional Patent		
☐ AP ARIPO Patent CH Ghana CM Camb KE Kenya LS Lesotho MW Malawi MZ Mozambique SD Sudan SL Sierra Leone SZ Swaziland TZ United Republic of Tanzania UG Uganda ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and the PCT		
☐ EA Eurasian Patent AM Armenia AZ Azerbaijan BY Belarus KG Kyrgyzstan KZ Kazakhstan MD Republic of Moldova RU Russian Federation TJ Tajikistan TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT		
☒ EP European Patent AT Austria BE Belgium CH & LI Switzerland and Liechtenstein CY Cyprus DE Germany DK Denmark ES Spain FI Finland FR France GB United Kingdom GR Greece IE Ireland IT Italy LU Luxembourg MC Monaco NL Netherlands PT Portugal SE Sweden TR Turkey and any other State which is a Contracting State of the European Patent Convention and of the ECT		
☐ OA OAPI Patent BF Burkina Faso BJ Benin CF Central African Republic CG Congo CI Cote d'Ivoire CM Cameroon CA Gabon GN Guinea GW Guinea-Bissau ML Mali MR Mauritania NE Niger SN Senegal TD Chad TG Togo and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)		
National Patent (if other kind of protection or treatment desired, specify on dotted line):		
☐ AE United Arab Emirates ☐ AG Antigua and Barbuda ☐ AL Albania ☐ AM Armenia ☐ AT Austria ☐ AU Australia ☐ AZ Azerbaijan ☐ BA Bosnia and Herzegovina ☐ BG Bulgaria ☐ BR Brazil ☐ BY Belarus ☐ BZ Belize ☒ CA Canada ☐ CH & LI Switzerland and Liechtenstein ☐ CN China ☒ CO Colombia ☐ CR Costa Rica ☐ CU Cuba ☐ CZ Czech Republic ☐ DE Germany ☐ DK Denmark ☐ DM Dominica ☐ DZ Algeria ☐ EE Estonia ☐ ES Spain ☐ FI Finland ☐ GB United Kingdom ☐ GD Grenada	☐ GE Georgia ☐ GH Ghana ☐ GM Gambia ☐ GR Greece ☐ HU Hungary ☐ ID Indonesia ☐ IL Israel ☐ IN India ☐ IS Iceland ☐ KE Kenya ☐ KG Kyrgyzstan ☐ KP Democratic People's Republic of Korea ☐ KR Republic of Korea ☐ KZ Kazakhstan ☐ LC Saint Lucia ☐ LK Sri Lanka ☐ LR Liberia ☐ LS Lesotho ☐ LT Lithuania ☐ LU Luxembourg ☐ LV Latvia ☐ MA Morocco ☐ MD Republic of Moldova ☐ MG Madagascar ☐ MK The former Yugoslav Republic of Macedonia ☐ MN Mongolia	☐ MW Malawi ☐ MX Mexico ☐ MZ Mozambique ☐ NO Norway ☐ NZ New Zealand ☐ PL Poland ☐ PT Portugal ☐ RO Romania ☐ RU Russian Federation ☐ SD Sudan ☐ SE Sweden ☐ SG Singapore ☐ SI Slovenia ☐ SK Slovakia ☐ SL Sierra Leone ☐ TJ Tajikistan ☐ TM Turkmenistan ☐ TR Turkey ☐ TT Trinidad and Tobago ☐ TZ United Republic of Tanzania ☐ UA Ukraine ☐ UG Uganda ☐ US United States of America ☐ UZ Uzbekistan ☐ VN Vietnam ☐ YU Yugoslavia ☐ ZA South Africa ☐ ZW Zimbabwe
Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet		
☐	☐	☐
☐	☐	☐
Precautionary Designation Statement In addition to the designations made above the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) not cited in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time. (Confirmation (marking fees) must reach the receiving Office within the 15-month time limit.)		

See also the PCT/CA form

31

PCT/CA 01/00837

5

Box No IX CHECK LIST LANGUAGE OF FILING																					
<i>The number of an application containing</i> (a) the following number of sheets in paper form <table style="width: 100%;"> <tr> <td style="width: 80%;">request (including declaration sheets)</td> <td style="width: 20%; text-align: right;">5</td> </tr> <tr> <td>sequence listing part</td> <td style="text-align: right;">-</td> </tr> <tr> <td>claims</td> <td style="text-align: right;">4</td> </tr> <tr> <td>abstract</td> <td style="text-align: right;">1</td> </tr> <tr> <td>drawings</td> <td style="text-align: right;">0</td> </tr> <tr> <td colspan="2"> </td> </tr> <tr> <td>Sub-total number of sheets</td> <td style="text-align: right;">(55) 60</td> </tr> <tr> <td>sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form see (b) below)</td> <td style="text-align: right;">10</td> </tr> <tr> <td colspan="2"> </td> </tr> <tr> <td>Total number of sheets</td> <td style="text-align: right;">(65) 70</td> </tr> </table> (b) sequence listing part of description filed in computer readable form (i) ☐ only (under Section 801(A)(i)) (ii) ☐ in addition to being filed in paper form (under Section 801(A)(ii)) Type and number of carriers (diskette CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 8(ii) in right column):	request (including declaration sheets)	5	sequence listing part	-	claims	4	abstract	1	drawings	0			Sub-total number of sheets	(55) 60	sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form see (b) below)	10			Total number of sheets	(65) 70	<i>The number of pp(s) in</i> item(s) (mark one applicable check-boxes below and indicate in right column the number of each item) 1 ☐ fee calculation sheet 2 ☐ original general power of attorney 4 ☐ copy of general power of attorney reference number if any 5 ☐ statement explaining lack of signature 6 ☐ priority document(s) identified in Box No. VI as item(s) 7 ☐ translation of international application into (language): 8 ☐ separate indications concerning deposited microorganism or other biological material 9 ☒ sequence listing in computer readable form (indicate also type and number of carriers (diskette CD-ROM CD-R or other)) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) (ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column 10 ☐ other (specify):
request (including declaration sheets)	5																				
sequence listing part	-																				
claims	4																				
abstract	1																				
drawings	0																				
Sub-total number of sheets	(55) 60																				
sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form see (b) below)	10																				
Total number of sheets	(65) 70																				
Figure of the drawings which should accompany the abstract 4	Language of filing of the international application. ENGLISH																				
Box No. X SIGNATURE OF APPLICANT AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>  Judy A. Erratt																					
<i>For receiving Office use only</i> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 60%; padding: 5px;"> 1 Date of actual receipt of the purported international application. 17 JUNE 2001 (11-06-01) </td> <td style="width: 40%; padding: 5px;"> 2 Drawings ☒ received. ☐ not received. </td> </tr> <tr> <td style="padding: 5px;"> 3 Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application </td> <td rowspan="2" style="padding: 5px;"> 6 ☒ Transmittal of search copy delayed until search fee is paid </td> </tr> <tr> <td style="padding: 5px;"> 4 Date of timely receipt of the required corrections under PCT Article 11(2) </td> </tr> <tr> <td style="padding: 5px;"> 5 International Searching Authority (if two or more are competent) ISA / </td> <td></td> </tr> </table> <i>For International Bureau use only</i> Date of receipt of the record copy by the International Bureau		1 Date of actual receipt of the purported international application. 17 JUNE 2001 (11-06-01)	2 Drawings ☒ received. ☐ not received.	3 Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	6 ☒ Transmittal of search copy delayed until search fee is paid	4 Date of timely receipt of the required corrections under PCT Article 11(2)	5 International Searching Authority (if two or more are competent) ISA /														
1 Date of actual receipt of the purported international application. 17 JUNE 2001 (11-06-01)	2 Drawings ☒ received. ☐ not received.																				
3 Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	6 ☒ Transmittal of search copy delayed until search fee is paid																				
4 Date of timely receipt of the required corrections under PCT Article 11(2)																					
5 International Searching Authority (if two or more are competent) ISA /																					

DELETED PICA

PCT/CA

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions Section 411)

RECEIVED

From the INTERNATIONAL BUREAU

To

OCT 25 2001

GOWLING

ERRATT Judy A.
Gowlings Laffleur Henderson
Suite 2600
180 Elgin Street
Ottawa Ontario K1P 1C3
ETATS-UNIS D AMERIQUE

Date of mailing (day/month/year) 15 October 2001 (15 10 01)	
Applicant's or agent's file reference 08-891816WO	IMPORTANT NOTIFICATION
International application No. PCT/CA01/00837	International filing date (day/month/year) 11 June 2001 (11 06 01)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 12 June 2000 (12 06 00)
Applicant UNIVERSITY OF SASKATCHEWAN	

- 1 The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, the International Bureau is in compliance with Rule 17 (a) or (b).
- 2 The asterisk (*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17 (a) or (b). In such a case, the attention of the applicant is directed to Rule 17 (c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- 3 The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau as provided by Rule 17 (a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17 (c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

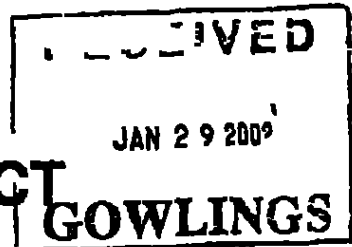
Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
12 June 2000 (12 06 00)	60/211,016	US	20 Sept 2001 (20 09 01)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740 14 35	Authorized officer P. Blanchet (Fax 338 87 40) Telephone No. (41-22) 338 83 38
--	--

form PCT/IS/304 (July 1998)

004370717

PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
Ernst Judy A
GOWLING LAFLEUR HENDERSON LLP
160 Elgin Street Suite 2600
Ottawa Ontario K1P 1C3
CANADA

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 39.3(c) and 61.1(b), first sentence
and Administrative Instructions Section 601(A))

Date of mailing
(day/month/year)

21 01 02

Applicant's or agent's file reference
no 891915W0

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/ 00837

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

- 1 The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application.

10/01/2002

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
☐ the actual date of receipt of the demand on behalf of this Authority (Rule 39.3(v)).
☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/M04), received the required corrections.

- 3 ☐ ATTENTION: That date of receipt is AFTER the expiration of 19 months from the priority date. Consequently the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 20 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 21). For details, see the PCT Applicant's Guide Volume II

- ☐ (If applicable) This notification confirms the information given by telephone facsimile transmission or in person

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

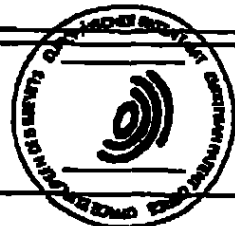


European Patent Office
D-80296 Munich
Tel (+49-89) 2399-0 Te 23456 epcpc e
Fax (+49-89) 2399-4465

Authorized officer:

BENKAARA A

Tel (+49-89) 2399-8865



Form PCT/IPEA/M02 (July 1998) P20432

(16/01/2002)

PATENT COOPERATION TREATY

34

PCT

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

From the INTERNATIONAL BUREAU

To

ERRATT, Judy A.
Gowlings Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1G3
CANADA

RECEIVED

GOWLING

Edwin

Date of mailing (day/month/year) 07 February 2002 (07 02 02)		IMPORTANT INFORMATION	
Applicant's or agent's file reference 08-891815WO			
International application No. PCT/CA01/00837	International filing date (day/month/year) 11 June 2001 (11 06 01)	Priority date (day/month/year) 12 June 2000 (12 06 00)	
Applicant UNIVERSITY OF SASKATCHEWAN			

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR
National: CA

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

National: CO

3. The applicant is reminded that he must enter the national phase before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 38(1)(a)) as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 38(3)(b) and Rule 74.1).

Some Offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Claudio BORTON
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/332 (September 1997)

4847689

PATENT COOPERATION TREATY

35

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:

GOWLING LAFLEUR HENDERSON LLP
Attn: Errat Judy A
160 Elgin Street Suite 2600
Ottawa Ontario K1P 1C3
CANADADate of mailing
(day/month/year)

24/04/2002

Applicant's or agent's file reference

08-891815W0

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/CA 01/00837

International filing date
(day/month/year)

11/06/2001

Applicant

UNIVERSITY OF SASKATCHEWAN

- 1
- ☒
- The Applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 18:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46).

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No., (41-22) 740.14.95

For more detailed instructions, see the notes on the accompanying sheet.

- ☐
- The applicant hereby declares that the International Search Report is not to be used for the purposes of Article 17(2)(a) to that effect is transmitted herewith.

- 3.
- ☐
- With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application or of the priority claim must reach the International Bureau as provided in Rules 80bis.1 and 80bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 30 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 18 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

European Patent Office, P.B. 5818 Postfach 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-3040 Tx. 31 851 apo nl
Fax. (+31-70) 340-3016

Authorized officer

Catherine Humbert

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, Article, Rule, and Section refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must accompany the amendments?

Letter (Section 205(b))

The amendments must be submitted with a letter

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 18(1)" (see below under "Statement under Article 18(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English. If the language of the international application is French, the letter must be in French.

37

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must in particular indicate in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped) whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter

1. [Where originally there were 48 claims and after amendment of some claims there are 51]
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]
a. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims:]
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added. or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where originally there were 13 claims and after amendment of all claims there are 21]
"Claims 1 to 13 cancelled; new claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 18 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.
(except that it may be in English or French or German)

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading preferably by using the words "Statement under Article 19(1)".

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations relevant to a given claim contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been filed, the applicant must preferably at the same time of filing the amendments with the International Preliminary Examining Authority (Rule 62.2(a) first sentence) also file a copy of such amendments with the International Preliminary Examining Authority.

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that where upon entry into the national phase, a translation of the claims as amended under Article 19 may also be furnished to the designated Offices (instead of, or in addition to, the translation of the claims as filed).

For further details on the requirements of each designated/elected Office see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

38

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 08-891815W0	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, Item 5 below	
International application No. PCT/CA 01/00837	International filing date (day/month/year) 11/06/2001	(Earliest) Priority Date (day/month/year) 12/06/2000
Applicant UNIVERSITY OF SASKATCHEWAN		

The International Search Report has been prepared by the International Searching Authority and is transmitted to the applicant.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence string

☒ contained in the international application in written form

☒ filed together with the international application in computer readable form

☐ furnished subsequently to this Authority in written form

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence string does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence string has been furnished.

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title

☒ the text is approved as submitted by the applicant.

☐ the text has been established by the Authority to read as follows.

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established according to Rule 38.2(b), by this Authority as it appears in Box I. The applicant may within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

4
☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 01/00837

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/09 A61K48/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

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

Electronic data base consulted during the international search phase or data base and, where practical, search terms used

EPO-Internal WPI Data BIOSIS MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Category of document and, if applicable, author, inventor, or the intended addressee	Relevance to the prior art
X	US 4 493 825 A (PLATT KENNETH B ET AL) 15 January 1985 (1985-01-15) column 1 line 11 - line 18 column 1 line 61 - column 2 line 66 column 3 line 48 - line 59 column 4 line 30 - line 41	1 5-7 19
X	US 5 328 987 A (MALISZEWSKI CHARLES R) 12 July 1994 (1994-07-12) column 2 line 11 - line 56 column 14 line 16 - column 15 line 12	1 5 6
	-/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in 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 document (as published on or after the international filing date)

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

O document relating to an oral disclosure (as evidenced by 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 documents of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is compared with one or more other such documents, each considered being relevant to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

12 April 2002

Date of issuing of the international search report

24/04/2002

Name and mailing address of the ISA

European Patent Office, P.O. Box 1
NL 2200 HV The Hague
Tel (+31-70) 340-2040 Telex 31 65 apo L
Fax (+31-70) 340-3016

Authorized officer

Montero Lopez B

Form PCT/ISA/210 (general version) (July 1992)

page 1 of 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 01/00837

YD

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of documents, with indication, where appropriate, of the relevant passages	Relevance to claim No.
X	M. YARNALL ET AL.: "Antibody response to Haemophilus somnus Fc receptor" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 27, no. 1, January 1989 (1989-01), pages 111-117, XP001004895 abstract page 111, right-hand column, paragraph 3 -page 112, left-hand column, paragraph 2	1,6
A	HANS JONSSON ET AL.: "The type-III Fc receptor from Streptococcus dysgalactiae is also an alpha2-macroglobulin receptor" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 220, 1994, pages 819-826, XP001064876 abstract; figure 1	1-27
A	JÓZSEF VASI ET AL.: "Five homologous repeats of the protein G-related protein MIG cooperate in binding to goat Immunoglobulin G" INFECTION AND IMMUNITY, vol. 67, no. 1, January 1999 (1999-01), pages 413-416, XP002195941 WASHINGTON US the whole document	1-27

21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 01/00837

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

The International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by the Authority, namely:
Although claims 19-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and the payment of additional search fees.

2

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/CA 01/00837

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
US 4493825	A	15-01-1985	AU	2129183 A		24-04-1984
			CA	1213828 A1		11-11-1986
			EP	0120955 A1		10-10-1984
			WO	8401289 A1		12-04-1984
US 5328987	A	12-07-1994	US	5198342 A		30-03-1993

13

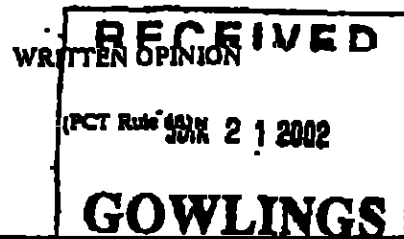
PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Errat, Judy A.
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C9
CANADA



Applicant's or agent's file reference 08-891815WC International application No.		REPLY DUE within 1 / 00 months/days from the above date of mailing, Priority date (day/month/year),
PCT/CA 01/ 00897	11/06/2001	12/06/2000
International Patent Classification (IPC) or both national classification and IPC C07K14/195		
Applicant UNIVERSITY OF SASKATCHEWAN		

- This written opinion is the first drawn up by this International Preliminary Examining Authority.
- This opinion contains indications relating to the following points:
 - ☒ Basis of the opinion
 - ☐ Priority
 - ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☐ Lack of unity of invention
 - ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; reasons and explanations supporting such statement
 - ☐ Certain documents cited
 - ☐ Certain defects in the international application
 - ☐ Certain observations on the international application
- The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

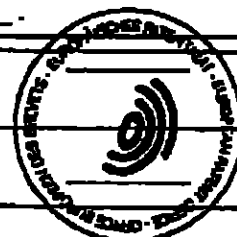
How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.5 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4ter. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
examination report must be established according to Rule 69.2 in: 12/10/2002

Name and mailing address of the IPEA/  European Patent Office D-80288 Munich Tel. (+49-89) 2399-0, Tx: 523656 epou d Fax: (+49-89) 2399-4435	Authorized office: Examiner Formalities officer (incl. extension of time limits) Tel. (+49-89) 2399 2838
--	--

Form PCT/IPEA/208 (cover sheet) (march 2002)



44

WRITTEN OPINION

International application NoPCT/CA 01/00837

I. Basis of the opinion

The basis of this written opinion is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been and will not be the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66.1(a) PCT; see also international search report).

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

1. To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:
2. In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims, which have been the subject of an international search report, does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim reference).
3. If amendments are filed, the applicant should comply with the requirements of Rule 66.8 PCT and indicate the basis of the amendments in the documents of the application as originally filed (Article 34 (2) (b) PCT) otherwise these amendments may not be taken into consideration for the establishment of the international preliminary examination report. The attention of the applicant is drawn to the fact that if the application contains an unnecessary plurality of independent claims, no examination of any of the claims will be carried out.

NB: Should the applicant decide to request detailed substantive examination, then an international preliminary examination report will normally be established directly. Exceptionally the examiner may draw up a second written opinion, should this be explicitly requested.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY.

PCT

To:

Ernst, Judy A.
GOWLING LAFLEUR HENDERSON LLP
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C3
CANADA

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

13/08/2002

Applicant's or agent's file reference

08-891815V0

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/00837

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

For the purpose of deciding whether the claimed invention is patentable or not, the elected Offices may apply criteria national to or different from the criteria on which the international preliminary examination report is based (see Articles 27 (5), 33 (5)). Additional criteria may include e.g. exemptions from patentability and the requirements of enabling disclosure and of clarity and support of claims.

Name and mailing address of the IPEA/

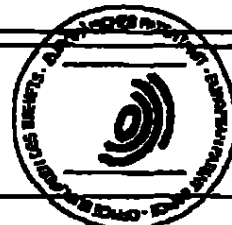


European Patent Office
D-80283 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epcan d
Fax: (+49-89) 2399-4465

Authorized officer

LLORCA A

Tel. (+49-89) 2399 2828



Form PCT/1PEA/116 (28/06/2002) P20473

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 08-891815W0	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPCBA/416)	
International application No. PCT/CA 01/ 00837	International filing date (day/month/year) 11/06/2001	Priority date (day/month/year) 12/06/2000
International Patent Classification (IPC) or national classification and IPC C07K14/195		
Applicant UNIVERSITY OF SASKATCHEWAN		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 2 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been appended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications relating to the following items:
- I ☒ Basis of the report
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; objections and explanations appearing on such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 10/01/2002	Date of completion of this report 08/08/2002
Name and mailing address of the IPEA/  European Patent Office Postfach 1015 69002 Heidelberg	Authorized officer BOUTRICHE J E E 114 1 11111 1111 1111

Form PCT/IPCBA/409 (cover sheet) (July 1998)



47

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application NoPCT/ CA 01/ 00837

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

III. non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (b) and Rule 66.1(e) PCT; see also international search report).

V. Reasoned statement under Rule 65.2(a)(II) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item II) above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims, which have been the subject of an international search report, does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim references).

PATENT COOPERATION TREATY

22800

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:

GOWLING LAFLEUR HENDERSON LLP
Attn. Erratt, Judy A.
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C3
CANADADate of mailing
(day/month/year)

01/03/2002

Applicant's or agent's file reference

08-891816W0

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/CA 01/00835

International filing date

(day/month/year)

11/06/2001

Applicant

UNIVERSITY OF SASKATCHEWAN

- 1.
- ☒
- The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 18:

The applicant is invited, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.95

For more detailed instructions, see the notes on the accompanying sheet.

- 2.
- ☐
- The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

- 3.
- ☐
- With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 10 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

Authorized officer

49

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the letter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 18 months from the priority date, whichever is earlier. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

52

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (j) the claim is cancelled;
- (k) the claim is new;
- (l) the claim replaces one or more claims as filed;
- (m) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 23, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1, 10 and 17 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claims 14, 15 and 16; claim 12 replaced by amended claim 12; claim 17 replaced by amended claim 17; claim 18 replaced by amended claim 18; claim 19 replaced by amended claim 19."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)".

It may not contain any disparaging comments on the international search report, or the relevance of citations contained in that report. References to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must, preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 52.2(a), first sentence).

Consequences with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/selected Office, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/selected Office, see Volume II of the PCT Applicant's Guide.

57

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or Agent's or Inventor's Name		FOR FURNISHING		see Instructions of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 6 above.
08-89181640		ACTION		
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)		
PCT/CA 01/00836	11/06/2001	12/06/2000		
Applicant				
UNIVERSITY OF SASKATCHEWAN				
This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau. This International Search Report comprises a total of <u>4</u> sheets. ☒ It is also accompanied by a copy of the prior art documents referred to in the report.				
1. Basis of the report 2. With regard to the language, the International search was carried out on the basis of the International application in the language in which it was filed, unless otherwise indicated under this item. ☐ the International search was carried out on the basis of a translation of the International application furnished to this Authority (Rule 33.1(b)). 3. With regard to any nucleotide and/or amino acid sequence disclosed in the International application, the International search was carried out on the basis of the sequence listing: ☒ contained in the International application in written form. ☒ filed together with the International application in computer readable form. ☐ furnished subsequently to this Authority in written form. ☐ furnished subsequently to this Authority in computer readable form. ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the International application as filed has been furnished. ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished. 4. ☒ Certain claims were found unsearchable (See Box I). 5. ☐ Unity of invention is lacking (see Box II). 6. With regard to the title, ☒ the text is approved as submitted by the applicant. ☐ the text has been established by this Authority to read as follows: 7. With regard to the abstract, ☒ the text is approved as submitted by the applicant. ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International search report, submit comments to this Authority. 8. The figure of the drawings to be published with the abstract is Figure No. <u>25</u> ☐ as suggested by the applicant ☐ None of the figures. ☐ because the applicant failed to suggest a figure. ☒ because this figure better characterizes the invention.				

52

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 01/00836

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K19/00 C12N15/62 C12N15/70 C12N1/21 A61K39/09
C07K16/12 G01N33/569 A61P31/04 A61K31/7088 G01N33/68

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)
IPC 7 C07K

Documentation searched other than patent documentation in the areas that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search words used)

BIOSIS, EMBASE, WPI, Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevance to claim No.
A	J. DALE: "Multivalent group A streptococcal vaccine designed to optimize the immunogenicity of six tandem M protein fragments." VACCINE, vol. 17, no. 2, January 1999 (1999-01), pages 193-200, XP004139975 Guildford, GB the whole document	1,6, 10-18, 20-22, 24-28, 33,34
A	EP 0 887 410 A (PFIZER INC.) 30 December 1998 (1998-12-30) page 6, line 46 -page 7, line 14 claims	1,6,7, 10-18, 20-34

-/-

☒ Further documents are listed in the continuation of part C.

☒ Patent family members are listed in 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 document but published on or after the international filing date
- *L* document which may throw doubts on priority claims or which is used to establish the publication date of another claim or other special reason (see specification)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *T* document published prior to the international filing date but later than the priority date claimed

- *T* prior document published after the international filing date or priority date and not in conflict with the application but used to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or original 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.
- *A* document member of the same patent family

Date of the actual completion of the international search

22 February 2002

Date of mailing of the international search report

01/03/2002

Name and mailing address of the ISA
European Patent Office, P.O. 5518 Patentplatz 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl
Fax (+31-70) 340-2010

Authorized officer

Noord, F

Form PCT/CA/218 (second sheet) (July 1992)

INTER: TIONAL SEARCH REPORT

International Application No.

PCT/CA 01/00836

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Content of document, with indication, where appropriate, of the relevant passages	Relevant to claim no.
A	WO 96 40928 A (IAF BIOVAC INC.) 19 December 1996 (1996-12-19) page 26, line 1 - line 21 claims 16, 5, 23E, 25, 28H	1, 6, 7, 10-22, 24-28, 33, 34
A	WO 98 18930 A (HUMAN GENOME SCIENCES INC.) 7 May 1998 (1998-05-07) seq. id. no. 54	8, 9
P, A	WO 00 39299 A (BIOCHEM PHARMA INC.) 6 July 2000 (2000-07-06) example 12 claims 21-32	1, 5-7, 10-16, 21, 22, 27, 28

Form PCT/CA 01/00836 (publication of search results) (July 2002)

27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 01/00836

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by the Authority, namely:
Although claims 27-32 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 8.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

J5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/CA 01/00836

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 887410	A	30-12-1998	AU 741724 B2 06-12-2001
			AU 7310098 A 24-12-1998
			BR 9802198 A 25-05-1999
			CA 2235471 A1 23-12-1998
			CN 1209455 A 03-03-1999
			EP 0887410 A2 30-12-1998
			JP 11103869 A 20-04-1999
WO 9640928	A	19-12-1996	US 5919620 A 06-07-1999
			AU 700080 B2 17-12-1998
			AU 5682896 A 30-12-1996
			BR 9609399 A 28-08-2001
			CA 2224015 A1 19-12-1996
			WO 9640928 A1 19-12-1996
			CN 1192241 A 02-09-1998
			CZ 9703942 A3 15-04-1998
			EP 0832238 A1 01-04-1998
			JP 11507214 T 29-06-1999
			NO 975752 A 06-02-1998
			PL 323781 A1 27-04-1998
			SK 168497 A3 08-07-1998
			TR 9701537 T1 21-03-1998
			ZA 9603987 A 31-10-1997
WO 9818930	A	07-05-1998	AU 5194598 A 22-05-1998
			AU 6909098 A 22-05-1998
			EP 0942983 A2 22-09-1999
			EP 0941335 A2 15-09-1999
			JP 2001505415 T 24-04-2001
			JP 2001501833 T 13-02-2001
			WO 9818930 A2 07-05-1998
			US 6159469 A 12-12-2000
WO 0039299	A	06-07-2000	AU 1764900 A 31-07-2000
			WO 0039299 A2 06-07-2000
			CN 1332803 T 23-01-2002
			CZ 20012161 A3 17-10-2001
			EP 1141306 A2 10-10-2001
			NO 20013045 A 20-08-2001

PATENT COOPERATION TREATY

12

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY**PCT RECEIVED**

Robins.

JAN 23 2002

To:

From: Judy A.
GOWLING LAFLEUR HENRIKSON LLP
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C3
CANADA

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY(PCT Rules 59.3(e) and 61.1(b), first sentences
and Administrative Instructions, Section 401(a))Date of mailing
(day/month/year)

17. 01. 02

Applicant's or agent's file reference

08-891616W0

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/ 00836

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

10/01/2002

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 18 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 30 months from the priority date (or later in some Offices) (Article 21). For details, see the PCT Applicant's Guide, Volume II.

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/



D-80398 Munich
Tel. (+49-89) 2199-0, Tx: 511696 optima d
Fax: (+49-89) 2199-4465

Authorized officer

BENKAABA A

Tel. (+49-89) 2199-8865



Form PCT/IPEA/402 (July 1998) P20452

(14/01/2002)

RECEIVED- Rolain's
FEB 13 2002**GOWLINGS****PATENT COOPERATION TREATY**

PCT

From the INTERNATIONAL BUREAU

To:

ERRATT, Judy, A.
Gowlings Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa, Ontario K1P 1C3
CANADA**INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION**

(PCT Rule 61.3)

Date of mailing (day/month/year) 25 January 2002 (25.01.02)		IMPORTANT INFORMATION
Applicant's or agent's file reference 08-891816WO		
International application No. PCT/CA01/00836	International filing date (day/month/year) 11 June 2001 (11.06.01)	Priority date (day/month/year) 12 June 2000 (12.06.00)
Applicant UNIVERSITY OF SASKATCHEWAN		

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR
National : CA

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

National : CO

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 38(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 38(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purpose of obtaining a European patent.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer: Telephone No. (41-22) 338.83.38
--	--

PATENT COOPERATION TREATY

RECEIVED

Robina
MAY 26 2002PCT
GOWLINGSFrom the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Erratt, Judy A.
GOWLING LAFLEUR HENDERSON LLP
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C3
CANADANOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

22/05/2002

Applicant's or agent's file reference
08-891816W0

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/00836

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/001).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

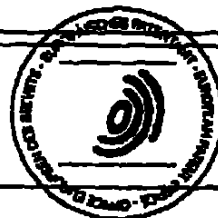


European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 521636 epma d
Fax (+49-89) 2399-4465

Authorized officer

BENKAABA A

Tel. (+49-89) 2399 3828



Form PCT/IPEA/416 (July 1992) P20473

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 08-891816W0	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/ISPA/416)	
International application No. PCT/CA 01/ 00836	International filing date (day/month/year) 11/06/2001	Priority date (day/month/year) 12/06/2000
International Patent Classification (IPC) or national classification and IPC C07K14/195		
Applicant UNIVERSITY OF SASKATCHEWAN		

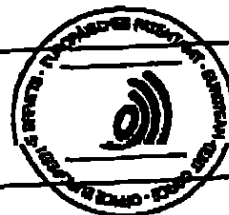
1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 2 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

1. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; comments and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 10/01/2002	Date of completion of this report 16/05/2002
Name and mailing address of the IPEA/  European Patent Office Tel. (+49-89) 2399-0, Telex 523646 eptou d Fax (+49-89) 2399-4465	Authorized officer BOUTRUCHE J I E Tel. (+49-89) 2399 2828



Form PCT/ISPA/409 (cover sheet) (July 1998)

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No PCT/CA 01/ 00836

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 86.1(e) PCT); see also international search report).

Summary of the examination under Rule 86.2(a)(II) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as defined in the claims, which have been the subject of an international search report, meets the criteria mentioned in Article 33(1) PCT, i.e. appears to be novel, to involve an inventive step and to be industrially applicable.

CORRECTED VERSION

RECEIVED

AGR - 8 2002

GOWLINGS

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 December 2001 (20.12.2001)

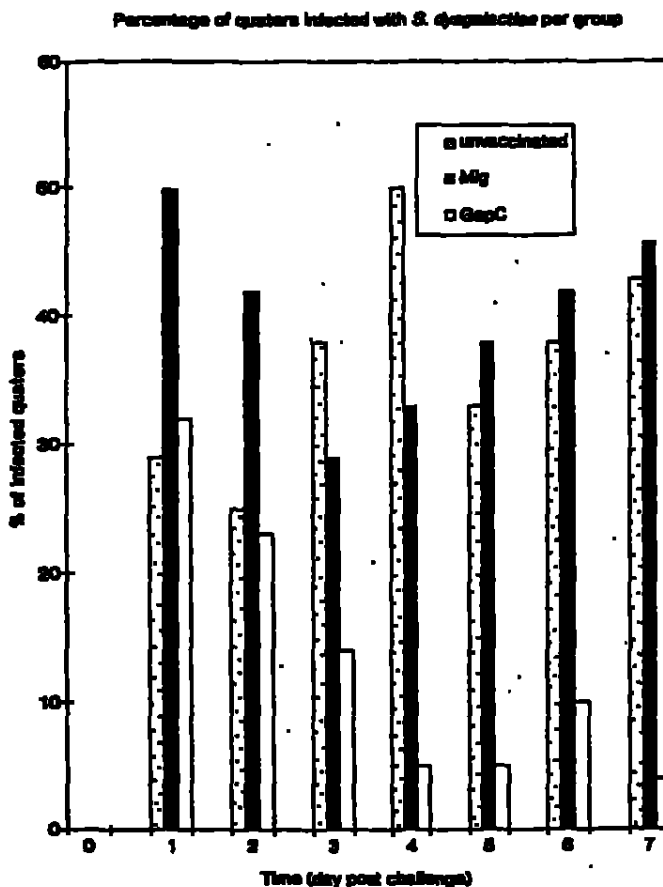
PCT

(10) International Publication Number
WO 01/96380 A2

- (51) International Patent Classification: C07K 14/195 (72) Inventors: POTTER, Andrew, A.; 521 Dalhousie Crescent, Saskatoon, Saskatchewan S7H 3S5 (CA). BOLTON, Alexandra, J.; 115-1540 29th Street N.W., Calgary, Alberta T2N 4M1 (CA). SONG, Xia, Ming; #8 1506 Main Street, Saskatoon, Saskatchewan S7H 0L7 (CA).
- (21) International Application Number: PCT/CA01/00837
- (22) International Filing Date: 11 June 2001 (11.06.2001)
- (25) Filing Language: English (74) Agents: ERRATT, Judy, A. et al.; Gowlings Lafleur Henderson LLP, Suite 2600, 160 Elgin Street, Ottawa, Ontario K1P 1C3 (CA).
- (26) Publication Language: English
- (30) Priority Data: 60/211,016 12 June 2000 (12.06.2000) US (81) Designated States (national): CA, CO.
- (71) Applicant: UNIVERSITY OF SASKATCHEWAN [CA/CA]; 120 Veterinary Road, Saskatoon, Saskatchewan S7N 5E3 (CA). (84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

[Continued on next page]

(54) Title: IMMUNIZATION OF DAIRY CATTLE WITH Mig PROTEIN



(57) Abstract: The Mig protein gene of *Streptococcus dysgalactiae*, and the corresponding amino acid sequence, is described, as is the use of the Mig protein in vaccine compositions to prevent and treat bacterial infections in general, and mastitis in particular.

WO 01/96380 A2

**Published:**

— *without international search report and to be republished -
upon receipt of that report*

(15) Information about Correction:

see PCT Gazette No. 12/2002 of 21 March 2002, Section
II

(48) Date of publication of this corrected version:

21 March 2002

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

63
62

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 December 2001 (20.12.2001)

PCT

(10) International Publication Number
WO 01/96380 A2

(51) International Patent Classification: C07K 14/195

Alexandra, J.; 115-1540 29th Street N.W., Calgary, Alberta T2N 4M1 (CA). SONG, Xia, Ming; #8 1506 Main Street, Saskatoon, Saskatchewan S7H 0L7 (CA).

(21) International Application Number: PCT/CA01/00837

(22) International Filing Date: 11 June 2001 (11.06.2001)

(74) Agents: ERRATT, Judy, A. et al.; Gowlings Lafleur Henderson LLP, Suite 2600, 160 Elgin Street, Ottawa, Ontario K1P 1C3 (US).

(25) Filing Language: English

(81) Designated States (national): CA, CO.

(26) Publication Language: English

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

(30) Priority Data:
60/211,016 12 June 2000 (12.06.2000) US

Published:
— without international search report and to be republished upon receipt of that report

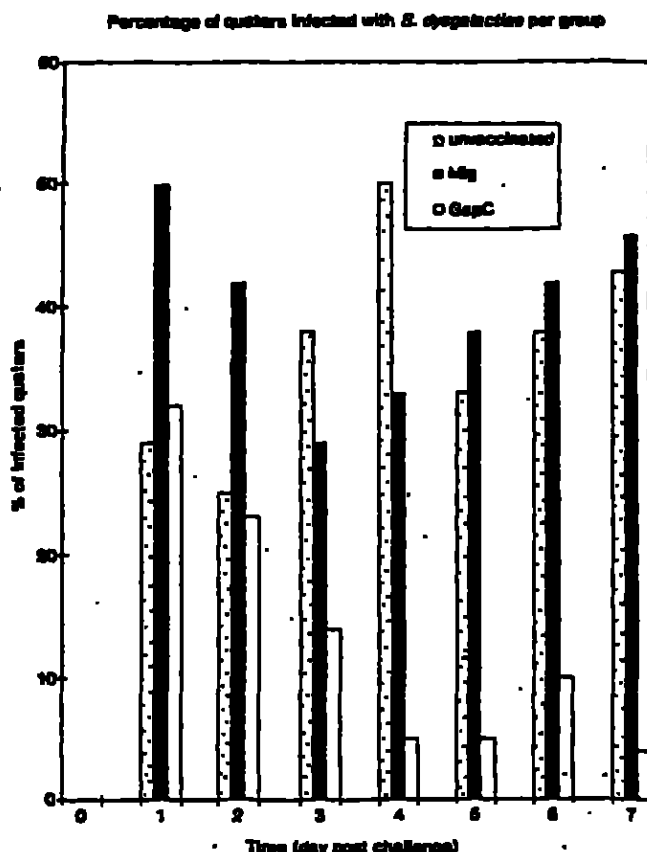
(71) Applicant: UNIVERSITY OF SASKATCHEWAN [CA/CA]; 120 Veterinary Road, Saskatoon, Saskatchewan S7N 5E3 (CA).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors: POTTER, Andrew, A.; 521 Dalhousie Crescent, Saskatoon, Saskatchewan S7H 3S5 (CA). BOLTON,

(54) Title: IMMUNIZATION OF DAIRY CATTLE WITH Mig PROTEIN

(57) Abstract: The Mig protein gene of *Streptococcus dysgalactiae*, and the corresponding amino acid sequence, is described, as is the use of the Mig protein in vaccine compositions to prevent and treat bacterial infections in general, and mastitis in particular.



WO 01/96380 A2

64
63

5

IMMUNIZATION OF DAIRY CATTLE WITH Mig PROTEIN

TECHNICAL FIELD

The present invention relates generally to bacterial antigens and genes encoding the same. More particularly, the present invention pertains to the cloning, expression and
10 characterization of the Mig Fc-receptor protein from several *Streptococcus* bacteria species, and the use of the same in vaccine compositions.

BACKGROUND

Mastitis is an infection of the mammary gland usually caused by bacteria or fungus.
15 The inflammatory response following infection results in decreased milk yield as well as quality, and causes major annual economic losses to the dairy industry.

Among the bacterial species most commonly associated with mastitis are various species of the genus *Streptococcus*, including *Streptococcus aureus*, *Streptococcus uberis* (untypeable), *Streptococcus agalactiae* (Lancefield group B), *Streptococcus dysgalactiae*
20 (Lancefield group C), *Streptococcus zooepidemicus*, and the Lancefield groups D, G, L and N streptococci. Some of those species are contagions (e.g. *S. agalactiae*), while others are considered environmental pathogens (e.g. *S. dysgalactiae* and *S. uberis*).

The environmental pathogen *S. uberis* is responsible for about 20% of all clinical cases of mastitis (Bramley, A.J. and Dodd, F.H. (1984) *J. Dairy Res.* 51:481-512; Bramley, A.J. (1987) *Animal Health Nutrition* 42:12-16; Watts, J.L. (1988) *J. Dairy Sci.* 71:1616-
25 1624); it is the predominant organism isolated from mammary glands during the non-lactating period (Bramley, A.J. (1984) *Br. Vet. J.* 140:328-335; Bramley and Dodd (1984) *J. Dairy Res.* 51:481-512; Oliver, S.P. (1988) *Am. J. Vet. Res.* 49:1789-1793).

Mastitis resulting from infection with *S. uberis* is commonly subclinical,
30 characterized by apparently normal milk with an increase in somatic cell counts due to the influx of leukocytes. The chemical composition of milk is changed due to suppression of secretion with the transfer of sodium chloride and bicarbonate from blood to milk, causing a shift of pH to a more alkaline level. *S. uberis* mastitis may also take the form of an acute

SUBSTITUTE SHEET (RULE 26)

clinical condition, with obvious signs of disease such as clots or discoloration of the milk and swelling or hardness of the mammary gland. Some cases of the clinical disease can be severe and pyrexia may be present. For a review of the clinical manifestations of *S. uberis* mastitis, see, Bramley (1991) Mastitis: physiology or pathology. p. 3-9. In C. Burvenich, G. Vandeputte-van Messom, and A. W. Hill (ed.), *New insights into the pathogenesis of mastitis*. Rijksuniversiteit Gent, Belgium; and Schalm et al. (1971) The mastitis complex-A brief summary. p. 1-3. In *Bovine Mastitis*. Lea & Febiger, Philadelphia.

Conventional antibacterial control methods such as teat dipping and antibiotic therapy are effective in the control of many types of contagious mastitis, but the environmental organisms typically found in all dairy barns are often resistant to such measures. Vaccination is therefore an attractive strategy to prevent infections of the mammary glands, and has been shown to be beneficial in the case of some contagious mastitis pathogens.

However, the literature is limited regarding vaccination studies with environmental pathogens such as *S. dysgalactiae* and *S. uberis*, and variable results have been observed. In some cases, immunization has resulted in increased sensitivity to the specific organism and in other cases strain-specific protection has been obtained.

For example, previous studies have shown that primary infection with *S. uberis* can considerably reduce the rate of infection following a second challenge with the same strain (Hill, A. W. (1988) *Res. Vet. Sci.* 44:386-387). Local vaccination with killed *S. uberis* protects the bovine mammary gland against intramammary challenge with the homologous strain (Finch et al. (1994) *Infect. Immun.* 62:3599-3603). Similarly, subcutaneous vaccination with live *S. uberis* has been shown to cause a dramatic modification of the pathogenesis of mastitis with the same strain (Hill et al. (1994) *FEMS Immunol. Med. Microbiol.* 8:109-118). Animals vaccinated in this way shed fewer bacteria in their milk and many quarters remain free of infection.

Nonetheless, vaccination with live or attenuated bacteria can pose risks to the recipient. Further, it is clear that conventional killed vaccines are in general largely ineffective against *S. uberis* and *S. agalactiae*, either due to lack of protective antigens on *in vitro*-grown cells or masking of these antigens by molecular mimicry.

The current lack of existing mastitis vaccines against *S. agalactiae* or the contagious streptococcus strains is due at least in part to a lack of knowledge regarding the virulence determinants and protective antigens produced by those organisms which are involved in

63
64

invasion and protection of the mammary gland (Collins et al. (1988) *J. Dairy Res.* 55:25-32; Leigh et al. (1990) *Res. Vet. Sci.* 49: 85-87; Marshall et al. (1986) *J. Dairy Res.* 53: 507-514).

S. dysgalactiae is known to bind several extracellular and plasma-derived proteins such as fibronectin, fibrinogen, collagen, alpha-II-macroglobulin, IgG, albumin and other compounds. The organism also produces hyaluronidase and fibrinolysin and is capable of adhering to and invading bovine mammary epithelial cells. However, the exact roles of the bacterial components responsible for these phenotypes in pathogenesis is not known.

Similarly, the pathogenesis of *S. uberis* infection is poorly understood. Furthermore, the influence of *S. uberis* virulence factors on host defense mechanisms and mammary gland physiology is not well defined. Known virulence factors associated with *S. uberis* include a hyaluronic acid capsule (Hill, A.W. (1988) *Res. Vet. Sci.* 45:400-404), hyaluronidase (Schaufuss et al. (1989) *Zentralbl. Bakteriол. Ser. A* 271:46-53), R-like protein (Groschup, M.H. and Timoney, J.F. (1993) *Res. Vet. Sci.* 54:124-126), and a cohemolysin, the CAMP factor, also known as UBERIS factor (Skalka, B. and Smola, J. (1981) *Zentralbl. Bakteriол. Ser. A* 249:190-194), R-like protein, plasminogen activator and CAMP factor. However, very little is known of their roles in pathogenicity.

The use of virulence determinants from *Streptococcus* as immunogenic agents has been proposed. For example, the CAMP factor of *S. uberis* has been shown to protect vertebrate subject from infection by that organism (Jiang, et al., U.S. Patent No. 5,863,543).

The γ antigen of the group B *Streptococci* strain A909 (ATCC No. 27591) is a component of the c protein marker complex, which additionally comprises an α and β subunit (Boyle, U.S. Patent No. 5,721,339). Subsets of serotype Ia, II, and virtually all serotype Ib cells of group B streptococci, have been reported to express components of the c protein. Use of the γ subunit as an immunogenic agent against infections by Lancefield Group B *Streptococcus* infection has been proposed. However, its use to prevent or treat bacterial infections in animals, including mastitis in cattle, has not been studied.

The group A streptococcal M protein is considered to be one of the major virulence factors of this organism by virtue of its ability to impede attack by human phagocytes (Lancefield, R.C. (1962) *J. Immunol.* 89:307-313). The bacteria persist in the infected tissue until antibodies are produced against the M molecule. Type-specific antibodies to the M protein are able to reverse the antiphagocytic effect of the molecule and allow efficient clearance of the invading organism.

M proteins are one of the key virulence factors of *Streptococcus pyogenes*, due to their involvement in mediating resistance to phagocytosis (Kehoe, M.A. (1991) *Vaccine* 9:797-806) and their ability to induce potentially harmful host immune responses via their superantigenicity and their capacity to induce host-cross-reactive antibody responses (Bisno, A.L. (1991) *New Engl. J. Med.* 325:783-793; Froude et al. (1989) *Curr. Top. Microbiol. Immunol.* 145:5-26; Stollerman, G.H. (1991) *Clin. Immunol. Immunopathol.* 61:131-142).

However, obstacles exist to using intact M proteins as vaccines. The protein's opsonic epitopes are extremely type-specific, resulting in narrow, type-specific protection. Further, some M proteins appear to contain epitopes that cross react with tissues of the immunized subject, causing a harmful autoimmune response (See e.g. Dale, J.G. and Beachey, E.H. (1982) *J. Exp. Med.* 156:1165-1176; Dale, J.B. and Beachey, E.H. (1985) *J. Exp. Med.* 161:113-122; Baird, R.W., Bronze, M.S., Draus, W., Hill, H.R., Veasey, L.G. and Dale, J.B. (1991) *J. Immun.* 146:3132-3137; Bronze, M.S. and Dale, J.B. (1993) *J. Immun.* 151:2820-2828; Cunningham, M.W. and Russell, S.M. (1983) *Infect. Immun.* 42:531-538).

Chimeric proteins containing three different fibronectin binding domains (FNBDs) derived from fibronectin binding proteins of *S. dysgalactiae* and *Staphylococcus aureus* have been expressed on the surface of *Staph. carnosus* cells. In the case of one of these proteins, intranasal immunizations with live recombinant *Staph. carnosus* cells expressing the chimeric protein on their surface resulted in an improved antibody response to a model immunogen present within the chimeric surface protein (Liljeqvist, S. et al. (1999) *FEBS Letters* 446:299-304).

Bacterial Fc receptors (surface moieties that bind to immunoglobulin molecules through a non-immune mechanism, i.e., to the Fc portion of the antibody) are a class of binding proteins further categorized by their reactivity with different classes and subclasses of mammalian immunoglobulins. The type I receptor (also known as Protein A), the most extensively studied and characterized, has been isolated from *Staphylococcus aureus*, and binds to IgG types 1,2 and 4; this receptor type further exhibits cross-reactivity with IgA and IgM. The type II Fc receptor, found on a few Lancefield Group A streptococci, and the type III receptor (also known as Protein G), found on the majority of human group C and group G strains of *streptococcus*, have been reported to react with all four types of IgG. In the case of the type III receptor, binding to IgG is highly specific; the protein does not cross-react with IgA or IgM. The type IV receptor is found in certain bovine group G streptococci, and the type V receptor is found on certain strains of *Streptococcus zooepidemicus*. The type VI

66
65

Fc receptor has been isolated from *S. zooepidemicus* strains S212 and RSS-212, and binds rat IgG with high affinity, i.e., 100 times that of Protein A binding, and 30 to 40 times as great as Protein G binding. (Boyle, et al., U.S. Patent No. 4,977,082). For a discussion of Fc receptors, see Langone (1982) *Adv. Immunol.* 32:167 and Myhre et al. (1984) *Basic Concepts of Streptococci and Streptococcal Diseases* (Hohn & Christensen, eds.) Redbook Ltd., Chertsey, Surrey, England.

Utility for Fc binding proteins have to date been limited to antibody detection and purification. With respect to clinical applications, a method of extracorporeal blood treatment of autoimmune disease which employs Fc binding proteins to remove antigen-antibody complexes has been proposed (see e.g. Fabnestock, U.S. Patent No. 4,954,618). However, their use in vaccine compositions has not previously been described nor suggested.

Until now, the protective capability of the *S. dysgalactiae* Mig protein against mastitis has not been studied, nor has the *S. dysgalactiae* Mig protein been isolated or characterized.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides Fc receptor proteins and uses therefor. In one embodiment, the invention is directed to a vaccine composition comprising a pharmaceutically acceptable vehicle and an Fc receptor protein. In certain embodiments, the Fc receptor protein is selected from the group consisting of:

- (a) a *Streptococcus dysgalactiae* Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4);
- (b) an Fc receptor protein having at least about 70% sequence identity to (a); and
- (c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

In some embodiments, the vaccine composition comprises an adjuvant.

In yet further embodiments, the invention is directed to a method of producing a vaccine composition. The method comprises the steps of

- (a) providing an Fc receptor protein or an immunogenic fragment thereof, the fragment comprising at least about 5 amino acids, and
- (b) combining said protein with a pharmaceutically acceptable vehicle.

In another embodiment, the invention is directed to a method of treating or preventing a bacterial infection in a vertebrate subject. The method comprises administering

to the subject a therapeutically effective amount of a vaccine composition as described above.

In certain embodiments the bacterial infection is a streptococcal infection. Additionally, the bacterial infection may cause mastitis.

5 In yet another embodiment, the invention is directed to a method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to the subject a therapeutically effective amount of a polynucleotide that comprises a coding sequence for an Fc receptor protein.

10 In certain embodiments, the bacterial infection is a streptococcal infection and may cause mastitis.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

15 Figures 1A-1D depict the polynucleotide sequence encoding the *Streptococcus dysgalactiae* Mig protein, and the amino acid sequence deduced therefrom (SEQ ID NO:3 and SEQ ID NO:4, respectively).

Figure 2 presents the following results for peptide structure analysis of the *S. dysgalactiae* Mig protein a: Kyle-Doolittle Hydropathy plot ("KD Hydrophilicity"), averaged
20 over a window of 7; an Emini surface probability plot ("Surface Prob."); a Karplus-Schulz chain flexibility plot ("Flexibility"); a Jameson-Wolf antigenic index plot, and both Chou-Fasman and Garnier-Osguthorpe-Robson secondary structure plots ("CF Alpha Helices" and "CF Beta Sheets"; and "GOR Turns," "GOR Alpha Helices," "GOR Beta Sheets," and "Glycosylation Sites," respectively).

25 Figure 3 is a Chou-Fasman secondary structure plot for the *S. dysgalactiae* Mig protein.

Figure 4 compares the change in the percentage of udder quarters infected with *S. dysgalactiae* over a 7 day period among three experimental groups: (1) unvaccinated control animals; (2) animals vaccinated with the Mig protein; and (3) animals vaccinated with GapC,
30 a plasmin binding protein isolated from *S. dysgalactiae* that was simultaneously evaluated. Infection was defined as recovery of >500 cfu of the *S. dysgalactiae* per ml of milk secretions.

Figure 5 depicts the observed inflammatory response to infection with *S. dysgalactiae* plotted as mean somatic cell counts (SCC) for each experimental group versus time in days post challenge. In the figure, diamonds (-♦-) represent unchallenged, unvaccinated quarters, squares (-■-) represent challenged, unvaccinated animals, triangles(-▲-) represent challenged, Mig-vaccinated animals, and x's (-X-) represent challenged, GapC-vaccinated animals.

Figure 6 illustrates somatic cell counts per mammary quarter on day 1 post-challenge. In the figure, the bar represents the mean for each group. Squares (-■-) represent unvaccinated animals; triangles (-▲-) represent GapC-vaccinated animals, and inverted triangles (-▼-) represent Mig-vaccinated animals.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Vols. I, II and III, Second Edition (1989); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications).

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A)	Arginine: Arg (R)
Asparagine: Asn (N)	Aspartic acid: Asp (D)
Cysteine: Cys (C)	Glutamine: Gln (Q)
Glutamic acid: Glu (E)	Glycine: Gly (G)
Histidine: His (H)	Isoleucine: Ile (I)
Leucine: Leu (L)	Lysine: Lys (K)
Methionine: Met (M)	Phenylalanine: Phe (F)
Proline: Pro (P)	Serine: Ser (S)
Threonine: Thr (T)	Tryptophan: Trp (W)
Tyrosine: Tyr (Y)	Valine: Val (V)

1. DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a *Streptococcus dysgalactiae* Fc binding protein" includes a mixture of two or more such proteins, and the like.

The terms "Fc receptor protein" and "Fc binding protein," used interchangeably herein, denote a bacterial protein capable of binding to immunoglobulin molecules at a site other than the antigen recognition site, including without limitation the Fc region of an immunoglobulin molecule.

The terms "Mig protein" and "Mig Fc receptor protein" and "Mig Fc binding protein" (used interchangeably herein) or a nucleotide sequence encoding the same, intends a protein or a nucleotide sequence, respectively, which is derived from a Mig gene found in a variety of bacterial species, including, without limitation, certain strains of group A streptococci. The nucleotide sequence of a representative *Streptococcus Mig* gene from *S. dysgalactiae* (SEQ ID NO:3), and the corresponding amino acid sequence encoded by that gene (SEQ ID NO:4), is depicted in Figures 1A-1D. However, a Mig protein as defined herein is not limited to the depicted sequences as subtypes of each of these *Streptococcus* species are known and variations in Mig proteins will occur between them.

A representative Mig gene derived from *S. dysgalactiae* is found in the plasmid pAA505Mig.

Furthermore, the derived protein or nucleotide sequences need not be physically derived from the gene described above, but may be generated in any manner, including for example, chemical synthesis, isolation (e.g., from *S. dysgalactiae*) or by recombinant production, based on the information provided herein. Additionally, the term intends proteins having amino acid sequences substantially homologous (as defined below) to contiguous amino acid sequences encoded by the genes, which display immunological and/or plasmin-binding activity.

Thus, the terms intend full-length, as well as immunogenic, truncated and partial sequences, and active analogs and precursor forms of the proteins. Also included in the term are nucleotide fragments of the gene that include at least about 8 contiguous base pairs, more preferably at least about 10-20 contiguous base pairs, and most preferably at least about 25 to

18
67

50, or more, contiguous base pairs of the gene, or any integers between these values. Such fragments are useful as probes and in diagnostic methods, discussed more fully below.

The terms also include those forms possessing, as well as lacking, a signal sequence, if such is present, as well as the nucleic acid sequences coding therefore. Additionally, the term intends forms of the Mig proteins which lack a membrane anchor region, and nucleic acid sequences encoding proteins with such deletions. Such deletions may be desirable in systems that do not provide for secretion of the protein. Furthermore, the Fc receptor-binding domains of the proteins, may or may not be present. Thus, for example, if the Mig Fc binding protein will be used to purify immunoglobulin, the Fc binding domain will generally be retained. If the protein is to be used in vaccine compositions, immunogenic epitopes which may or may not include the Fc receptor binding domain, will be present.

The terms also include proteins in neutral form or in the form of basic or acid addition salts depending on the mode of preparation. Such acid addition salts may involve free amino groups and basic salts may be formed with free carboxyls. Pharmaceutically acceptable basic and acid addition salts are discussed further below. In addition, the proteins may be modified by combination with other biological materials such as lipids (both those occurring naturally with the molecule or other lipids that do not destroy immunological activity) and saccharides, or by side chain modification, such as acetylation of amino groups, phosphorylation of hydroxyl side chains, oxidation of sulfhydryl groups, glycosylation of amino acid residues, as well as other modifications of the encoded primary sequence.

The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic - aspartate and glutamate; (2) basic - lysine, arginine, histidine; (3) non-polar - alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar - glycine, asparagine, glutamine, cystine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence

SUBSTITUTE SHEET (RULE 26)

as the reference molecule, but possessing minor amino acid substitutions that do not substantially affect the immunogenicity and/or plasmin-binding affinity of the protein, are therefore within the definition of the reference polypeptide.

For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 or 20-50 conservative or non-conservative amino acid substitutions, or any integer between these values, so long as the desired function of the molecule remains intact.

Similarly, substitutions occurring in the transmembrane binding domain, if present, and the signal sequence, if present, normally will not affect immunogenicity. One of skill in the art may readily determine other regions of the molecule of interest that can tolerate change by reference to the peptide structure plots shown in Figure 2 and Figure 3 herein.

The term "streptococcal Mig protein" intends a Mig Fc binding protein, as defined above, derived from a streptococcal species that produces the same, including, but not limited to *S. dysgalactiae*. For example, a "*S. dysgalactiae* Mig protein" is a Fc binding protein as defined above, derived from *S. dysgalactiae*.

"Wild type" or "native" proteins or polypeptides refer to proteins or polypeptides isolated from the source in which the proteins naturally occur. "Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

An "isolated" protein or polypeptide is a protein or polypeptide molecule separate and discrete from the whole organism with which the molecule is found in nature; or a protein or polypeptide devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith.

The term "functionally equivalent" intends that the amino acid sequence of a Mig Fc binding protein is one that will elicit a substantially equivalent or enhanced immunological response, as defined above, as compared to the response elicited by a Fc binding protein having identity with the reference Fc binding protein, or an immunogenic portion thereof.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." Antibodies that recognize the same epitope can be identified

68

in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

The terms "immunogenic" protein or polypeptide refer to an amino acid sequence which elicits an immunological response as described below. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the Fc receptor protein in question, with or without the signal sequence, membrane anchor domain and/or Fc binding domain, analogs thereof, or immunogenic fragments thereof.

By "immunogenic fragment" is meant a fragment of an Fc receptor protein which includes one or more epitopes and thus elicits the immunological response described below. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., *Epitope Mapping Protocols* in *Methods in Molecular Biology*, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Patent No. 4,708,871; Geysen et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:3998-4002; Geysen et al. (1986) *Molec. Immunol.* 23:709-715.

Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols*, *supra*. Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots, such as those calculated using, e.g., the Omega version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al., *Proc. Natl. Acad. Sci. USA* (1981) 78:3824-3828 for determining antigenicity profiles, and the Kyte-Doolittle technique, Kyte et al., *J. Mol. Biol.* (1982) 157:105-132 for hydropathy plots. Figures 2 and 3 herein depict Kyte-Doolittle profiles for representative proteins encompassed by the invention.

Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, preferably at least about 5 amino acids, more preferably at least about 10-15 amino acids, and most preferably 25 or more amino acids, of the parent Mig Fc receptor protein molecule. There is no critical upper limit to the length of the fragment,

which may comprise nearly the full-length of the protein sequence, or even a fusion protein comprising two or more epitopes of Mig.

An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

By "subunit vaccine composition" is meant a composition containing at least one immunogenic polypeptide, but not all antigens, derived from or homologous to an antigen from a pathogen of interest. Such a composition is substantially free of intact pathogen cells or particles, or the lysate of such cells or particles. Thus, a "subunit vaccine composition" is prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or recombinant analogs thereof. A subunit vaccine composition can comprise the subunit antigen or antigens of interest substantially free of other antigens or polypeptides from the pathogen.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or $\gamma\delta$ T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance of the mammary gland to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host and/or a quicker recovery time.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal

administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

5 The term "treatment" as used herein refers to either (1) the prevention of infection or reinfection (prophylaxis), or (2) the reduction or elimination of symptoms of the disease of interest (therapy).

By "mastitis" is meant an inflammation of the mammary gland in mammals, including in cows, ewes, goats, sows, mares, and the like, caused by the presence of *S. uberis*. The infection manifests itself by the infiltration of phagocytic cells in the gland. Generally, 4 clinical types of mastitis are recognized: (1) peracute, associated with swelling, heat, pain, and abnormal secretion in the gland and accompanied by fever and other signs of systemic disturbance, such as marked depression, rapid weak pulse, sunken eyes, weakness and complete anorexia; (2) acute, with changes in the gland similar to those above but where fever, anorexia and depression are slight to moderate; (3) subacute, where no systemic changes are displayed and the changes in the gland and its secretion are less marked: and (4) subclinical, where the inflammatory reaction is detectable only by standard tests for mastitis.

Standard tests for the detection of mastitis include but are not limited to, the California Mastitis Test, the Wisconsin Mastitis Test, the Nagase test, the electronic cell count and somatic cell counts used to detect a persistently high white blood cell content in milk. In general, a somatic cell count of about 300,000 to about 500,000 cells per ml or higher, in milk will indicate the presence of infection. Thus, a vaccine is considered effective in the treatment and/or prevention of mastitis when, for example, the somatic cell count in milk is retained below about 500,000 cells per ml. For a discussion of mastitis and the diagnosis thereof, see, e.g., *The Merck Veterinary Manual: A Handbook of Diagnosis, Therapy, and Disease Prevention and Control for the Veterinarian*, Merck and Co., Rahway, New Jersey, 1991.

By the terms "vertebrate," "subject," and "vertebrate subject" are meant any member of the subphylum Chordata, including, without limitation, mammals such as cattle, sheep, pigs, goats, horses, and humans; domestic animals such as dogs and cats; and birds, including domestic, wild and game birds such as cocks and hens including chickens, turkeys and other gallinaceous birds; and fish. The term does not denote a particular age. Thus, both adult and newborn animals, as well as fetuses, are intended to be covered.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

5 An "isolated" nucleic acid molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature; or a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith. The term "isolated" in the context of a polynucleotide intends that the
10 polynucleotide is isolated from the chromosome with which it is normally associated, and is isolated from the complete genomic sequence in which it normally occurs.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is
15 naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

A "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a
20 nucleotide sequence which is transcribed and translated into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory elements. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from
25 eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence. A "complementary" sequence is one in which the nitrogenous base at a given nucleotide position is the complement of the nitrogenous base appearing at the same position in the reference sequence. To illustrate, the complement of adenosine is thymosine, and vice versa; similarly,
30 cytosine is complementary to guanine, and vice versa; hence, the complement of the reference sequence 5'-ATGCTGA-3' would be 5'-TACGACT-3'.

27
70

A "wild-type" or "native" sequence, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., the *S. dysgalactiae* Mig protein coding sequences depicted in Figures 1A-1D (SEQ ID NO:4).

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

"Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis, such as ALIGN, Dayhoff, M.O. in *Atlas of Protein Sequence and Structure* M.O.

Dayhoff ed., 5 Suppl. 3:353-358, National biomedical Research Foundation, Washington, DC, which adapts the local homology algorithm of Smith and Waterman (1981) *Advances in Appl. Math.* 2:482-489 for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.nih.gov/BLAST/>.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of

77
71

the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning, supra*; *Nucleic Acid Hybridization, supra*.

By the term "degenerate variant" is intended a polynucleotide containing changes in the nucleic acid sequence thereof, that encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the polynucleotide from which the degenerate variant is derived.

Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a bacterial gene, the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacteria. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A vector is capable of transferring gene sequences to target cells (e.g., bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers, and liposomes).

Typically, the terms "vector construct," "expression vector," "gene expression vector," "gene delivery vector," "gene transfer vector," and "expression cassette" all refer to an assembly which is capable of directing the expression of a sequence or gene of interest. Thus, the terms include cloning and expression vehicles, as well as viral vectors.

These assemblies include a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. The expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

DNA "control elements" refers collectively to transcription promoters, transcription enhancer elements, transcription termination sequences; polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), translation termination sequences, upstream regulatory domains, ribosome binding sites and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. See e.g., McCaughan et al. (1995) *PNAS USA* 92:5431-5435; Kochetov et al (1998) *FEBS Letts.* 440:351-355. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter and the coding sequence and the promoter can still be considered "operably linked" to the coding sequence. Similarly, "control elements compatible with expression in a subject" are those which are capable of effecting the expression of the coding sequence in that subject.

A control element, such as a promoter, "directs the transcription" of a coding sequence in a cell when RNA polymerase will bind the promoter and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed, or is capable of transformation, by an exogenous nucleic acid molecule.

23
72

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, external secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, organs, biopsies and also samples of *in vitro* cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, e.g., recombinant cells, and cell components.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (e.g., biotin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. Particular examples of labels which may be used under the invention include fluorescein, rhodamine, dansyl, umbelliferone, Texas red, luminol, NADPH and α - β -galactosidase.

2. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

Central to the present invention is the discovery that the Mig Fc binding protein isolated from *S. dysgalactiae* is capable of eliciting an immune response in a vertebrate subject. In particular, the gene for the Mig protein in *S. dysgalactiae* has been isolated, sequenced and characterized, and the amino acid sequence encoded by the gene has been deduced therefrom. The complete DNA sequence for the *S. dysgalactiae* mig gene (SEQ ID NO:3) and the corresponding amino acid sequence (SEQ ID NO:4) are shown in Figures 1A-1D.

As described in the examples, the full-length *S. dysgalactiae* mig gene, depicted at nucleotide positions 1-2,010 inclusive, of Figures 1A-1D, encodes the full-length *S. dysgalactiae* Mig protein of 669 amino acids, which includes a first 24 amino acid transmembrane region (residues 15 and 39) and a second 18 amino acid transmembrane region of 18 amino acids (residues 646-664). Transmembrane regions were determined using the TMPred program, which predicts membrane-spanning regions and their orientation. The program uses an algorithm based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins, using a combination of several weight matrices for scoring. See Hofmann, K., & Stoffel, W. (1993) *Biol. Chem.* 347:166.

S. dysgalactiae Mig has a predicted molecular weight of about 73 kDa. (calculated using the Peptide Sort program of the GCG Wisconsin Package, version 10, provided by the SeqWeb sequence analysis package, version 1.1 of the Canadian Bioinformatics Resource). The full-length sequence includes a signal peptide.

Figure 2 plots the following structural analyses results for the Mig protein of the present invention: Kyte-Doolittle hydrophathy, averaged over a window of 7; surface probability according to Emami; chain flexibility according to Karplus-Schulz; antigenicity index according to Jameson-Wolf; secondary structure according to Garnier-Osguthorpe-Robson; secondary structure according to Chou-Fasman; and predicted glycosylation sites. Figure 3 plots secondary structure according to Chou-Fasman for the Mig protein of the present invention. One of skill in the art can readily use the information presented in Figures 2 and 3 to determine immunogenic regions in the protein for use in vaccine compositions.

Mig Fc receptor proteins, including without limitation the *S. dysgalactiae* Mig protein, immunogenic fragments thereof or chimeric proteins including the same, can be

-24
73

provided in subunit vaccine compositions. In addition to use in vaccine compositions, the proteins or antibodies thereto can be used as diagnostic reagents to detect the presence of infection in a vertebrate subject. Similarly, the genes encoding the proteins can be cloned and used to design probes to detect and isolate homologous genes in other bacterial strains.

5 For example, fragments comprising at least about 15-20 nucleotides, more preferably at least about 20-50 nucleotides, and most preferably about 60-100 nucleotides, or any integer between these values, will find use in these embodiments.

The vaccine compositions of the present invention can be used to treat or prevent a wide variety of bacterial infections in vertebrate subjects. For example, vaccine
10 compositions including, without limitation, the Mig Fc receptor protein from *S. dysgalactiae*, can be used to treat streptococcal infections in vertebrate subjects that are caused by this or other species. In particular, *S. uberis*, *S. agalactiae*, and *S. dysgalactiae* are common bacterial pathogens associated with mastitis in bovine, equine, ovine and goat species. Additionally, group B streptococci, such as *S. agalactiae*, are known to cause numerous
15 other infections in vertebrates, including septicemia, meningitis, bacteremia, impetigo, arthritis, urinary tract infections, abscesses, spontaneous abortion etc. Hence, vaccine compositions containing the Mig Fc receptor protein will find use in treating and/or preventing a wide variety of streptococcal infections.

Similarly, Fc binding proteins derived from other bacterial genera such as
20 *Staphylococcus* will find use for treating bacterial infections caused by species belonging to those genera. Thus, it is readily apparent that Fc binding proteins from a variety of bacterial species can be used to treat and /or prevent a wide variety of bacterial infections in numerous animal species.

The streptococcal Fc binding proteins of the present invention, including without
25 limitation the Mig Fc binding protein, can be used in vaccine compositions either alone or in combination with other bacterial, fungal, viral or protozoal antigens. These antigens can be provided separately or even as fusion proteins comprising one or more epitopes of an Fc binding protein fused to one or more of these antigens. For example, other immunogenic proteins from *S. uberis*, such as the CAMP factor, hyaluronic acid capsule, hyaluronidase, R-
30 like protein and plasminogen activator, can be administered with the Mig protein. Additionally, immunogenic proteins from other organisms involved in mastitis, such as from the genera *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Nocardia*, *Clostridium*, *Mycobacterium*, *Mycoplasma*, *Pasteurella*, *Prototheca*, other streptococci, coliform bacteria,

as well as yeast, can be administered along with the Fc binding proteins described herein to provide a broad spectrum of protection. Thus, for example, immunogenic proteins from *Staphylococcus aureus*, *Str. agalactiae*, *Str. dysgalactiae*, *Str. zooepidemicus*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, *Clostridium*
5 *perfringens*, *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella spp.* can be provided along with the Fc binding proteins of the present invention.

Additionally, Fc proteins from different streptococcal species may be used together in the vaccine compositions of the present invention. In this embodiment, the multiple Fc proteins may be provided either as fusion proteins or as discrete antigens in the same or
10 different vaccine compositions.

Production of Fc Binding Proteins

The above-described Fc binding proteins and active fragments, analogs and chimeric proteins derived from the same, can be produced by variety of methods. Specifically, Fc
15 binding proteins can be isolated directly from bacteria which express the same. This is generally accomplished by first preparing a crude extract which lacks cellular components and several extraneous proteins. The desired proteins can then be further purified from the cell lysate fraction by, e.g., column chromatography, HPLC, immunoabsorbent techniques or other conventional methods well known in the art.

More particularly, techniques for isolating Fc binding proteins have been described. For example, the methods of Reis, et al. have been used to isolate a functionally
20 homogeneous Fc receptor having the properties of a type-III receptor (Reis et al., (1984) *J. Immunol.* 132:3091).

Alternatively, the proteins can be recombinantly produced as described herein. As
25 explained above, these recombinant products can take the form of partial protein sequences, full-length sequences, precursor forms that include signal sequences, mature forms without signals, or even fusion proteins (e.g., with an appropriate leader for the recombinant host, or with another subunit antigen sequence for *Streptococcus* or another pathogen).

In one embodiment of the present invention, the *S. dysgalactiae* Mig protein is
30 purified from a cell lysate fraction using affinity chromatography after recombinantly producing the protein. See Example 1A-D, *infra*.

25
74

Gene libraries can be constructed and the resulting clones used to transform an appropriate host cell. Colonies can be pooled and screened for clones having Fc receptor binding activity, i.e., for clones capable of binding IgG.

Alternatively, once the amino acid sequences are determined, oligonucleotide probes which contain the codons for a portion of the determined amino acid sequences can be prepared and used to screen genomic or cDNA libraries for genes encoding the subject proteins. The basic strategies for preparing oligonucleotide probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e.g., *DNA Cloning*, Vol. 11, *supra*; *Nucleic Acid Hybridization*, *supra*; *Oligonucleotide Synthesis*, *supra*; Sambrook et al., *supra*. Once a clone from the screened library has been identified by positive hybridization, it can be confirmed by restriction enzyme analysis and DNA sequencing that the particular library insert contains a Fc binding protein gene or a homolog thereof. The genes can then be further isolated using standard techniques and, if desired, PCR approaches or restriction enzymes employed to delete portions of the full-length sequence.

Similarly, genes can be isolated directly from bacteria using known techniques, such as phenol extraction and the sequence further manipulated to produce any desired alterations. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA.

Alternatively, DNA sequences encoding the proteins of interest can be prepared synthetically rather than cloned. The DNA sequences can be designed with the appropriate codons for the particular amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.

Once coding sequences for the desired proteins have been prepared or isolated, they can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and

Bacillus subtilis), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), YCp19 (*Saccharomyces*) and bovine papilloma virus (mammalian cells). See, Sambrook et al., *supra*; *DNA Cloning, supra*; B. Perbal, *supra*.

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. If a signal sequence is included, it can either be the native, homologous sequence, or a heterologous sequence. For example, the signal sequence for the *S. dysgalactiae* Mig protein (amino acid residue 1 to 39, inclusive) can be used for secretion thereof, as can a number of other signal sequences, well known in the art. Leader sequences can be removed by the host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the proper reading frame. It may also be desirable to produce mutants or analogs of the Fc binding protein. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are described in, e.g., Sambrook et al., *supra*; *DNA Cloning, supra*; *Nucleic Acid Hybridization, supra*.

The expression vector is then used to transform an appropriate host cell. A number of mammalian cell lines are known in the art and include immortalized cell lines available

76
75

from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guilliermondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*.

Depending on the expression system and host selected, the proteins of the present invention are produced by culturing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into the growth media, the protein can be purified directly from the media. If the protein is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The proteins of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis, using known amino acid sequences or amino acid sequences derived from the DNA sequence of the genes of interest. Such methods are known to those skilled in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra*, Vol. 1, for classical solution synthesis. Chemical synthesis of peptides may be preferable if a small fragment of the antigen in question is capable of raising an immunological response in the subject of interest.

The Fc binding proteins of the present invention, or their fragments, can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal, (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an antigen of the

present invention, or its fragment, or a mutated antigen. Serum from the immunized animal is collected and treated according to known procedures. See, e.g., Jurgens et al. (1985) *J. Chrom.* 348:363-370. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by immunoaffinity chromatography, using known procedures.

5 Monoclonal antibodies to the Mig protein and to the fragments thereof, can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by using hybridoma technology is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g.,
10 M. Schreier et al., *Hybridoma Techniques* (1980); Hammerling et al., *Monoclonal Antibodies and T-cell Hybridomas* (1981); Kennett et al., *Monoclonal Antibodies* (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,452,570; 4,466,917; 4,472,500, 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against the Mig protein, or fragments thereof, can be screened for various properties; i.e., for isotype, epitope,
15 affinity, etc. Monoclonal antibodies are useful in purification, using immunoaffinity techniques, of the individual antigens which they are directed against. Both polyclonal and monoclonal antibodies can also be used for passive immunization or can be combined with subunit vaccine preparations to enhance the immune response. Polyclonal and monoclonal antibodies are also useful for diagnostic purposes.

Vaccine Formulations and Administration

The Fc binding proteins of the present invention can be formulated into vaccine compositions, either alone or in combination with other antigens, for use in immunizing subjects as described below. Methods of preparing such formulations are described in, e.g.,
25 *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 18 Edition, 1990. Typically, the vaccines of the present invention are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in or suspension in liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active
30 immunogenic ingredient is generally mixed with a compatible pharmaceutical vehicle, such as, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents.

27
76

Adjuvants which enhance the effectiveness of the vaccine may also be added to the formulation. Adjuvants may include for example, muramyl dipeptides, avridine, aluminum hydroxide, dimethyldioctadecyl ammonium bromide (DDA), oils, oil-in-water emulsions, saponins, cytokines, and other substances known in the art.

5 The Mig protein may be linked to a carrier in order to increase the immunogenicity thereof. Suitable carriers include large, slowly metabolized macromolecules such as proteins, including serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well known to those skilled in the art; polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like; 10 polymeric amino acids such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles.

The Fc binding proteins of the present invention may be used in their native form or their functional group content may be modified by, for example, succinylation of lysine residues or reaction with Cys-thiolactone. A sulfhydryl group may also be incorporated into 15 the carrier (or antigen) by, for example, reaction of amino functions with 2-iminothiolane or the N-hydroxysuccinimide ester of 3-(4-dithiopyridyl) propionate. Suitable carriers may also be modified to incorporate spacer arms (such as hexamethylene diamine or other bifunctional molecules of similar size) for attachment of peptides.

Other suitable carriers for the Fc binding proteins of the present invention include 20 VP6 polypeptides of rotaviruses, or functional fragments thereof, as disclosed in U.S. Patent No. 5,071,651. Also useful is a fusion product of a viral protein and the subject immunogens made by methods disclosed in U.S. Patent No. 4,722,840. Still other suitable carriers include cells, such as lymphocytes, since presentation in this form mimics the natural mode of presentation in the subject, which gives rise to the immunized state. Alternatively, the 25 proteins of the present invention may be coupled to erythrocytes, preferably the subject's own erythrocytes. Methods of coupling peptides to proteins or cells are known to those of skill in the art.

Furthermore, the Fc binding proteins (or complexes thereof) may be formulated into vaccine compositions in either neutral or salt forms. Pharmaceutically acceptable salts 30 include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example,

sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Vaccine formulations will contain a "therapeutically effective amount" of the active ingredient, that is, an amount capable of eliciting an immune response in a subject to which the composition is administered. In the treatment and prevention of mastitis, for example, a "therapeutically effective amount" would preferably be an amount that enhances resistance of the mammary gland to new infection and/or reduces the clinical severity of the disease. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered somatic cell count in milk from the infected quarter. For example, the ability of the composition to retain or bring the somatic cell count (SCC) in milk below about 500,000 cells per ml, the threshold value set by the International Dairy Federation, above which, animals are considered to have clinical mastitis, will be indicative of a therapeutic effect.

The exact amount is readily determined by one skilled in the art using standard tests. The Fc binding protein concentration will typically range from about 1% to about 95% (w/w) of the composition, or even higher or lower if appropriate. With the present vaccine formulations, 5 to 500 µg of active ingredient per ml of injected solution should be adequate to raise an immunological response when a dose of 1 to 3 ml per animal is administered.

To immunize a subject, the vaccine is generally administered parenterally, usually by intramuscular injection. Other modes of administration, however, such as subcutaneous, intraperitoneal and intravenous injection, are also acceptable. The quantity to be administered depends on the animal to be treated, the capacity of the animal's immune system to synthesize antibodies, and the degree of protection desired. Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves. The subject is immunized by administration of the vaccine in at least one dose, and preferably two doses. Moreover, the animal may be administered as many doses as is required to maintain a state of immunity to infection.

Additional vaccine formulations which are suitable for other modes of administration include suppositories and, in some cases, aerosol, intranasal, oral formulations, and sustained release formulations. For suppositories, the vehicle composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about 1% to about 2%. Oral vehicles include such normally

28
77

employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and contain from about 10% to about 5 95% of the active ingredient, preferably about 25% to about 70%.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and 10 benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the protein into carriers or vehicles such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as 15 hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. The Fc binding proteins can also be delivered using implanted mini-pumps, well known in the art.

The Fc binding proteins of the instant invention can also be administered via a carrier virus which expresses the same. Carrier viruses which will find use with the instant 20 invention include but are not limited to the vaccinia and other pox viruses, adenovirus, and herpes virus. By way of example, vaccinia virus recombinants expressing the novel proteins can be constructed as follows. The DNA encoding the particular protein is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to 25 transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the instant protein into the viral genome. The resulting TK recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

An alternative route of administration involves gene therapy or nucleic acid 30 immunization. Thus, nucleotide sequences (and accompanying regulatory elements) encoding the subject Fc binding proteins can be administered directly to a subject for *in vivo* translation thereof. Alternatively, gene transfer can be accomplished by transfecting the subject's cells or tissues *ex vivo* and reintroducing the transformed material into the host.

DNA can be directly introduced into the host organism, i.e., by injection (see International Publication No. WO/90/11092; and Wolff et al. (1990) *Science* 247:1465-1468). Liposome-mediated gene transfer can also be accomplished using known methods. See, e.g., Hazinski et al. (1991) *Am. J. Respir. Cell Mol. Biol.* 4:206-209; Brigham et al. (1989) *Am. J. Med. Sci.* 298:278-281; Canonico et al. (1991) *Clin. Res.* 39:219A; and Nabel et al. (1990) *Science* 249:1285-1288. Targeting agents, such as antibodies directed against surface antigens expressed on specific cell types, can be covalently conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells susceptible to infection.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 10801 University Boulevard, Manassas. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid, made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for a period of thirty (30) years from the date of deposit. The organisms will be made available by the ATCC under the terms of the Budapest Treaty, which assures permanent and unrestricted availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. §122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. §1.12 with particular reference to 886 OG 638). Upon the granting of a patent, all restrictions on the availability to the public of the deposited cultures will be irrevocably removed.

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35 U.S.C. §112. The nucleic acid sequences of these genes, as well as the amino acid sequences of the molecules encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the description herein.

29
78

5	Bacterial Strain	Plasmid	Gene	Deposit Date	ATCC No.
	DE5a	pAA505Mig	<i>S. dysgalactiae</i> mig gene	May 31, 2000	PTA-1977

EXPERIMENTAL

10 Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be
15 allowed for.

Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

20 **Materials and Methods**

Enzymes were purchased from commercial sources, and used according to the manufacturers' directions.

In the isolation of DNA fragments, except where noted, all DNA manipulations were done according to standard procedures. See, Sambrook et al., *supra*. Restriction enzymes,
25 T₄ DNA ligase, *E. coli*, DNA polymerase I, Klenow fragment, and other biological reagents can be purchased from commercial suppliers and used according to the manufacturers' directions. Double stranded DNA fragments were separated on agarose gels.

EXAMPLE 1**Preparation, Amplification, Sequencing, Expression, Purification and Characterization of the *S. dysgalactiae* Mig Fc Receptor Protein****A. Preparation of *S. dysgalactiae* Chromosomal DNA**

5 A clinical *S. dysgalactiae* isolate from a case of bovine mastitis (ATCC Accession No. 43078) was obtained from the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209), and was used as a source of DNA. The organism was routinely grown on TSA sheep blood agar plates (PML Microbiologicals, Mississauga, Ontario) at 37° C for 18 hours, or in Todd-Hewitt broth (Oxoid Ltd., Hampshire, England) supplemented with 0.3% yeast extract (Sigma, St. Louis, Missouri) (THB-YE) at 37° C, 5% CO₂.

10 Chromosomal DNA was prepared from *S. dysgalactiae* grown in 100 ml of THB-YE supplemented with 20 mM glycine for approximately 6 hours, until an A₆₀₀ of 0.8 to 1.0 was reached. Cells were harvested and re-suspended in 50 mM EDTA, 50 mM Tris-HCl, 0.5% TWEEEN-20 (Sigma, St. Louis, MO) and supplemented with RNase A (200 mg/ml), proteinase K (20 mg/ml), lysozyme (100 mg/ml) and mutanolysin (100 mg/ml). (Sigma, St. Louis, Missouri). Following bacterial lysis for 30 minutes at 37° C with vigorous shaking, guanidine hydrochloride and TWEEEN-20, pH 5.5, were mixed with the lysate to give a final concentration of 0.8 M and 5%, respectively. This mixture was incubated at 50° C for 30 minutes. The chromosomal DNA was then purified using a Qiagen genomic-tip 100g (Qiagen, Germany) and precipitated using 0.7 volumes of isopropanol. The resulting pellet was washed in 70% ethanol and re-suspended in 0.5 ml 10 mM Tris-HCl, pH 8.8.

B. Amplification and Cloning of the *S. dysgalactiae* Mig Gene

25 The *Mig* gene was amplified by PCR (See Mullis et al., U.S. Patent No. 4,683,195; Mullis, U.S. Patent No. 4,683,202;) using the forward primer *mig1* (SEQ ID NO:1, shown in Table 1) and the reverse primer *mig1r* (SEQ ID NO:2, shown in Table 1). In the sequences depicted in Table 1, underlining denotes nucleotides added to the original sequence, and bolding indicates the location of restriction endonuclease recognition sites.

80
79

TABLE 1
Tabulation of Sequences

SEQUENCE ID NO:	SEQUENCE NAME	NUCLEOTIDE/AMINO ACID SEQUENCE
1	Primer <i>mig1</i>	5'- <u>G</u> <u>CGG</u> <u>CCA</u> <u>TGG</u> TAG AAA ATA CTA TAA CTG-3'
2	Primer <i>mig1R</i>	5'- <u>ACG</u> <u>CCC</u> <u>GGG</u> TTA GTC TTC TTT ACG TTT-3'
3	<i>Streptococcus dysgalactiae</i> strain SDG8 <i>mig</i> gene	(see Figure 1)
4	<i>Streptococcus dysgalactiae</i> strain SDG8 Mig protein	
5	Primer <i>mig-3</i>	5'- GTT GGC CTA GAT ATC ACA GAA TTA CAA -3'
6	Primer <i>mig-4</i>	5'- AAA GCA CCC GGG CCA GCC ATT ACT G -3'
7	Primer <i>mig-6</i>	5'- AGG TGC TTC CCA TGG AAC TGC CTG AAC T -3'
8	Primer <i>mig-7</i>	5'- GGC GAG AGT CTA GAA ACT AAA GCG AAA AAC -3'
9	Primer <i>mig-8</i>	5'- GCA ATC ACC AGG ATC CTC AGT AAC CAT TTC -3'
10	Primer <i>mig-9</i>	5'- CAG GCA GTT CAT ATG GAA GCA CCT ACA GT -3'
11	Primer <i>mig-10</i>	5'- TCC CGG AGT AGC ATT GTC AGT C -3'
12	Primer <i>mig-11</i>	5'- GCA GCG GTC CAT ATG CCT GTT GGC CTA GAT -3'
13	Primer <i>mig-12</i>	5'- GCC TGA ACT GGA TCC CTC AAC TGA TCT G -3'
14	Primer <i>mig-13</i>	5'- TTC CGT TGG ATC CTG CAA CTC CAA TTG -3'
15	Primer <i>mig-14</i>	5'- TAA GTC AAA AGC TTT GAC AAT TAG TCT T -3'

PCR was carried out using Vent DNA polymerase (New England Biolabs, Mississauga, Ontario, Canada). 0.7 µg of *S. dysgalactiae* genomic DNA, 1 µM each of the *mig1* (SEQ ID NO:1) *mig1r* (SEQ ID NO:2) primers (see above), 200 µM each of dATP, dCTP, dGTP and dTTP, 3mM MgSO₄, 1X ThermoPol PCR buffer (New England Biolabs), and 2 units of Vent DNA Polymerase were combined. The reaction mix was then incubated for 3 cycles of 1 minute at 94°C, 3 minutes at 50°C, and 1 minute, 10 seconds at 72°C, followed by 27 cycles of 15 seconds at 95°C, 30 seconds at 55°C, 1 minute at 72°C, followed by a single cycle of 5 minutes at 72°C.

The *mig* PCR product obtained above and the expression vector pAA505 (VIDO, Saskatoon, Saskatchewan, Canada) were digested with the restriction enzymes *NcoI* and *SmaI* (Amersham Pharmacia, Quebec, Canada) according to the manufacturer's instructions, and the *mig* sequence was ligated into the same sites of the vector.

This construct was used to transform *E. coli* DH5 α (Life Technologies, Gaithersburg, MD). The transformed *E. coli* DH5 α cells bearing the pAA505-*mig* vector construct were designated *E. coli* DH5α pAA505Mig.

C. Nucleotide sequence of the *S. dysgalactiae mig* Gene and the Corresponding Deduced Amino Acid Sequence

The nucleotide sequence in both orientations of the *mig* gene was determined on an ABI 373 DNA automatic sequencer (Applied Biosystems) at the Plant Biotechnology Institute (PBI, Saskatoon, Saskatchewan, Canada) by using the multiple primers shown in Table 1 (Primers *mig-2* through *mig-14*).

Figures 1A-1D depict the nucleotide coding sequence and the amino acid sequence, respectively for the *S. dysgalactiae* Mig protein (SEQ ID NO:3 and SEQ ID NO:4, respectively).

These sequences were then compared against known sequences via BLAST analysis. The search parameters used to analyze the nucleic acid sequence were as follows: Database: nt; Number of letters in database: 1,961,177,913; Number of sequences in database: 614,801; Matrix: blastn matrix:1-3; Gap Penalties: Existence: 5, Extension: 2. The results obtained showed that the *S. dysgalactiae* SD8 *mig* gene depicted in Figures 1A-1D is homologous to several known nucleotide sequences, e.g., there is 98% homology with the *mig* gene for *S. dysgalactiae* SC1 (Emb Accession No. Z29666.1 SDMIGSUP).

84
80

The search parameters used to analyze the amino acid sequence were as follows:

Database: nr; Number of letters in database: 157,988,256; Number of Sequences in database: 503,479; Matrix: BLOSUM62; Gap Penalties: Existence:11, Extension: 1. The results obtained showed that the *S. dysgalactiae* SD8 mig amino acid sequence depicted in Figures 1A-1D is up to 89% homologous to several known amino acid sequences.

D. Expression and Purification of the Recombinant *S. dysgalactiae* Mig Protein

E. coli containing the construct prepared in Example 1B, *supra*, was grown in LB broth containing 100 µg/ml ampicillin to an A_{600} of approximately 0.5. Expression of the Mig protein was then induced by the addition of 1 mM isopropyl-β,D-thiogalactoside (IPTG) (Sigma, St. Louis, MO). Following three hours incubation at 37°C, cells were harvested, washed in column buffer (50mM sodium phosphate pH 8.0, 0.2 M NaCl) and lysed by sonication.

Approximately 40% of the recombinant protein was in the soluble fraction of the cell sonicate with a yield of approximately 50 mg of the recombinant protein per litre of culture volume, as determined using a DC Protein Assay kit (BioRad Laboratories, Mississauga, Ontario, Canada) with bovine serum albumin (Pierce, Rockford, Illinois) as a standard.

The recombinant Mig protein was purified by affinity chromatography using an agarose-IgG matrix, based upon the protein's ability to bind the Fc portion of the IgG molecule. The cell lysate was cleared by centrifugation and the soluble fraction was applied to a BLIgG agarose column (Sigma, St. Louis, Missouri). The column was washed with 10 column volumes of column buffer (50 mM sodium phosphate pH 8.0, 0.3 M NaCl), and the protein eluted with column buffer plus 0.1 M glycine, pH 2.5, yielding a homogeneous protein fraction with a Mig concentration of approximately 10-15 mg/ml. The eluate was dialyzed against 2,000 volumes of PBSA and stored at -20°C.

E. Characterization of the Recombinant Protein

Analysis of the purified protein by SDS-PAGE demonstrated 60% purity.

EXAMPLE 2

Immunization with Mig and experimental infection of cattle

Vaccines were formulated to contain 50 mg/ml of affinity purified recombinant Mig or GapC in the oil-based adjuvant VSA3 (VIDO, Saskatoon, Saskatchewan, Canada). VSA3 is a combination of Emulsigen Plus™ (MVP Laboratories, Ralston, Nebraska) and Dimethyldioctadecyl ammonium bromide (Kodak, Rochester, New York)..

24 non-lactating Holsteins with no history of *S. dysgalactiae* infection were obtained from various farms in Saskatchewan, Canada. One week prior to vaccination, all animals were treated with Cephadry™ (300 mg per quarter; Ayerst Laboratories, Montreal, Canada), in order to clear any infection of the udders prior to the vaccination step.

Three groups of eight animals were immunized subcutaneously with two doses of vaccines containing Mig, GapC (a plasmin binding protein isolated from streptococcus bacteria that was evaluated simultaneously), or a placebo with a three-week interval between immunizations. Two weeks following the second immunization, animals were exposed to 650 colony forming units of *S. dysgalactiae* delivered into three quarters with an udder infusion cannula. The fourth quarter on each animal served as an un-infective control.

All animals were examined daily for clinical signs of disease and samples from all udder quarters were collected on each day. Samples were observed for consistency and somatic cell counts as well as bacterial numbers were determined.

EXAMPLE 3

Bacterial Colonization

Bacteria were enumerated by spreading serial dilutions (10^8 to 10^3) directly onto TSA sheep blood agar plates followed by overnight incubation at 37°C, 5% CO₂. Colonization is defined as >500 cfu/ml of the challenge organism recovered.

To confirm that the bacteria recovered from milk secretions were *S. dysgalactiae*, selected colonies recovered from each animal were tested using an API strep-20 test (bioMerieux SA, Hazelwood, Missouri) according to the manufacturer's instructions. This test is a standardized method which combines 20 biochemical assays for enzymatic activity and sugar fermentation, the results of which yield an analytical profile. The profile permits identification of the particular streptococcal species present by either referring to an analytical profile index or using identification software.

Following challenge with *S. dysgalactiae*, animals from all groups were shown to be colonized by *S. dysgalactiae* (Figure 4). The Mig-immunized animals showed a reduction in the number of infected quarters on days three and four post-challenge.

87
81**EXAMPLE 4****Measuring Inflammatory Response**

Inflammatory response was measured as a function of somatic cell count (i.e., lymphocytes, neutrophils, and monocytes). Somatic cell counts were measured in a Coulter counter using standard techniques, as recommended by Agriculture and Agri-Food Canada Pamphlet IDF50B (1985) *Milk and Milk products—Methods of Sampling*. Samples were always read within 48 hours of collection and fixation.

The number of somatic cells present in the gland was determined at days 1 through 7 post-challenge. Numbers from the unchallenged quarter remained constant throughout the trial, while on day 1, the Mig-immunized group was lower than the placebo-immunized group (Figure 5). The individual data from day 1 is shown in Figure 6.

Thus, the cloning, expression and characterization of the *S. dysgalactiae* Mig protein are disclosed, as are methods of using the same.

Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

CLAIMSWhat is claimed is:

1. A vaccine composition comprising a pharmaceutically acceptable vehicle and an
5 Fc receptor protein.

2. The vaccine composition of claim 1, wherein said Fc receptor protein is selected
from the group consisting of:

- 10 (a) a *Streptococcus dysgalactiae* Mig protein comprising the amino acid sequence
shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4);
(b) an Fc receptor protein having at least about 70% sequence identity to (a); and
(c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5
amino acids.

15 3. The vaccine composition of claim 2, wherein said Mig protein comprises the
amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid
positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4) or an immunogenic fragment
thereof, said fragment comprising at least about 5 amino acids.

20 4. The vaccine composition of claim 3, wherein said Mig protein comprises the
amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid
positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4).

25 5. The vaccine composition of claim 1, further comprising an adjuvant.

6. A method of producing a vaccine composition comprising the steps of
(a) providing an Fc receptor protein or an immunogenic fragment thereof, said
fragment comprising at least about 5 amino acids, and
(b) combining said protein with a pharmaceutically acceptable vehicle.

30

83
82

7. Use of an Fc receptor protein for the manufacture of a vaccine composition useful for treating or preventing a bacterial infection in a vertebrate subject.

8. The use according to claim 7, wherein the Fc receptor protein is selected from the group consisting of:

- (a) a *Streptococcus dysgalactiae* Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4);
- (b) an Fc receptor protein having at least about 70% sequence identity to (a); and
- (c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

9. The use according to claim 8, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

10. The use according to claim 9, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4).

11. The use according to any of claims 7-10, wherein said bacterial infection is a streptococcal infection.

12. The use according to claim 11, wherein said bacterial infection causes mastitis.

13. Use of a polynucleotide comprising a coding sequence for an Fc receptor protein in the manufacture of a medicament useful for treating or preventing a bacterial infection in a vertebrate subject.

14. The use according to claim 13, wherein the Fc receptor protein is selected from the group consisting of:

- (a) a *Streptococcus dysgalactiae* Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4);

(b) an Fc receptor protein having at least about 70% sequence identity to (a); and
(c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

5 15. The use according to claim 14, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

10 16. The use according to claim 15, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4).

15 17. The use according to any of claims 13-16, wherein said bacterial infection is a streptococcal infection.

18. The use according to claim 17, wherein said bacterial infection causes mastitis.

20 19. A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to any of claims 1-5.

25 20. The method of claim 19 wherein said bacterial infection is a streptococcal infection.

21. The method of claim 20 wherein said bacterial infection causes mastitis.

30 22. A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a polynucleotide, said polynucleotide comprising a coding sequence for an Fc receptor protein.

84
83

23. The method of claim 22, wherein the Fc receptor protein is selected from the group consisting of:

(a) a *Streptococcus dysgalactiae* Mig protein comprising the amino acid sequence shown at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4);

5 (b) an Fc receptor protein having at least about 70% sequence identity to (a); and

(c) immunogenic fragments of (a) and (b), said fragments comprising at least about 5 amino acids.

10 24. The method of claim 23, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids.

15 25. The method of claim 24, wherein said Mig protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* Mig protein depicted at amino acid positions 1 to 669, inclusive, of Figures 1A-1D (SEQ ID NO:4).

20 26. The method of any of claims 22-25, wherein said bacterial infection is a streptococcal infection.

27. The method of claim 26, wherein said bacterial infection causes mastitis.

85
84

1/9

atg gaa aaa gaa aaa aaa gta aaa tac ttt tta cgt aaa tca gct ttt	48
Met Glu Lys Glu Lys Lys Val Lys Tyr Phe Leu Arg Lys Ser Ala Phe	
1 5 10 15	
gga tta gcg tct gta tca gct gcg ttt tta gtt tog gga gca cta gaa	96
Gly Leu Ala Ser Val Ser Ala Ala Phe Leu Val Ser Gly Ala Leu Glu	
20 25 30	
aat act ata act gtt tct gca gaa act ata cct gca gcg gtc att gta	144
Asn Thr Ile Thr Val Ser Ala Glu Thr Ile Pro Ala Ala Val Ile Val	
35 40 45	
cct gtt ggc cta gat act aca gaa tta caa aaa tgg tat gac att gca	192
Pro Val Gly Leu Asp Thr Thr Glu Leu Gln Lys Trp Tyr Asp Ile Ala	
50 55 60	
aat gat tta gtt gcg act gac aat gct act ccg gga ggc gta ttt aca	240
Asn Asp Leu Val Ala Thr Asp Asn Ala Thr Pro Gly Gly Val Phe Thr	
65 70 75 80	
gca gac tca atg aag gca tta tat cgt tta cta aat gat gca tac gat	288
Ala Asp Ser Met Lys Ala Leu Tyr Arg Leu Leu Asn Asp Ala Tyr Asp	
85 90 95	
gtg ttg gaa tca aaa gac tat aga aaa tat gat tct caa gat agg att	336
Val Leu Glu Ser Lys Asp Tyr Arg Lys Tyr Asp Ser Gln Asp Arg Ile	
100 105 110	
gtt gaa ttg gta aac aat tta aag aat act acg cag tct ctt tta cca	384
Val Glu Leu Val Asn Asn Leu Lys Asn Thr Thr Gln Ser Leu Leu Pro	
115 120 125	
att gga gta gaa cca gta gta ttt gat act act cgc ttg aat acc tgg	432
Ile Gly Val Glu Pro Val Val Phe Asp Thr Thr Arg Leu Asn Thr Trp	
130 135 140	
tat gat gct gct aat gaa att gtt aat aat tca gat gct tat aca gca	480
Tyr Asp Ala Ala Asn Glu Ile Val Asn Asn Ser Asp Ala Tyr Thr Ala	
145 150 155 160	
gaa tca att cag tog ttg tat aag tta att aat gat gca tac gat gtg	528
Glu Ser Ile Gln Ser Leu Tyr Lys Leu Ile Asn Asp Ala Tyr Asp Val	
165 170 175	
tta gaa tca aaa gat tac agt aag tat gat tct caa gat aaa gtc aac	576
Leu Glu Ser Lys Asp Tyr Ser Lys Tyr Asp Ser Gln Asp Lys Val Asn	
180 185 190	
aat ctt gca gat cag ttg aga gat gca gtt cag gca gtt caa cta gaa	624
Asn Leu Ala Asp Gln Leu Arg Asp Ala Val Gln Ala Val Gln Leu Glu	
195 200 205	

FIG. 1A

SUBSTITUTE SHEET (RULE 26)

Peptide Structure Results

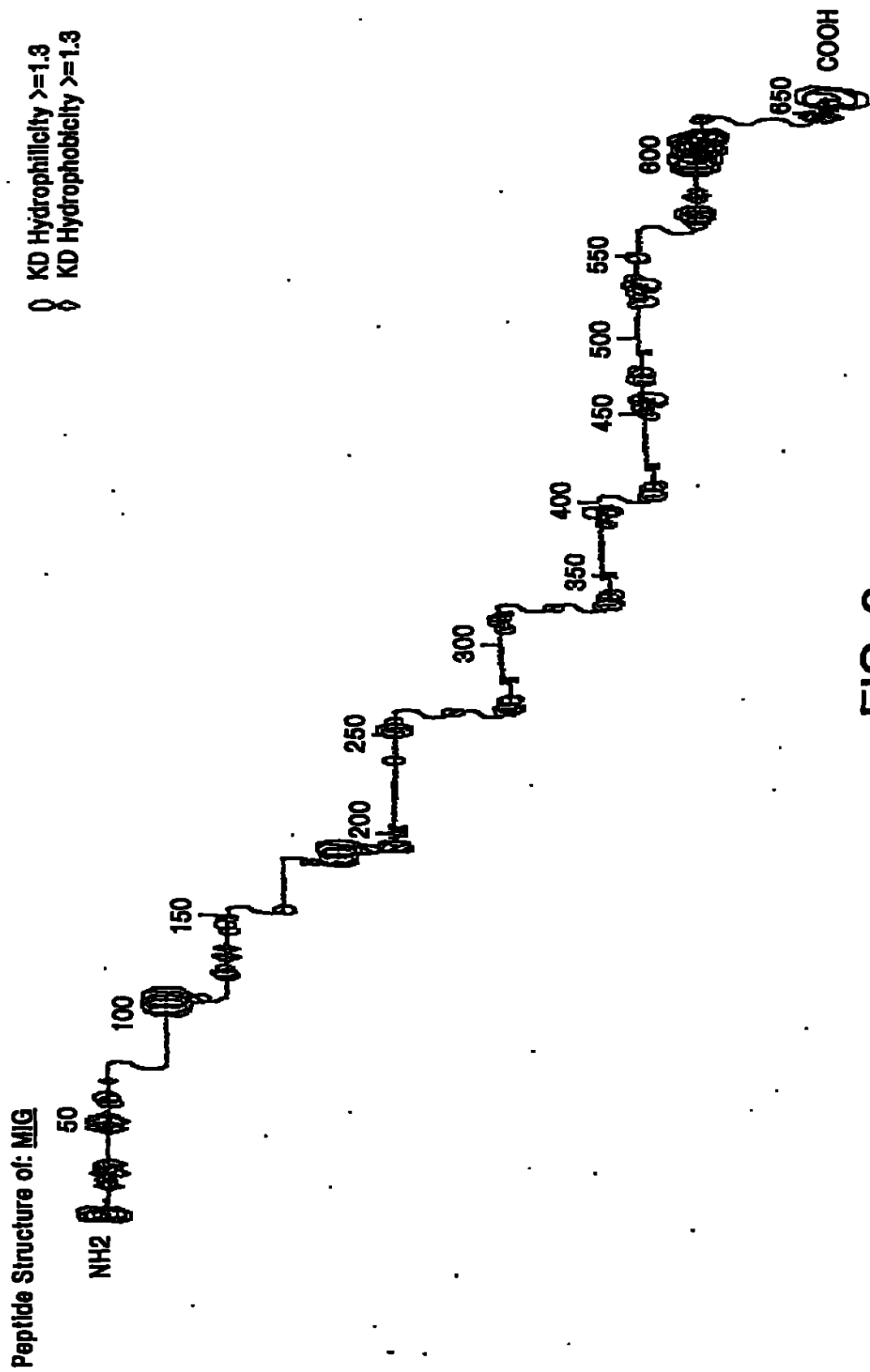


FIG. 3

8/9

Mean Somatic Cell Counts (SCC) per group

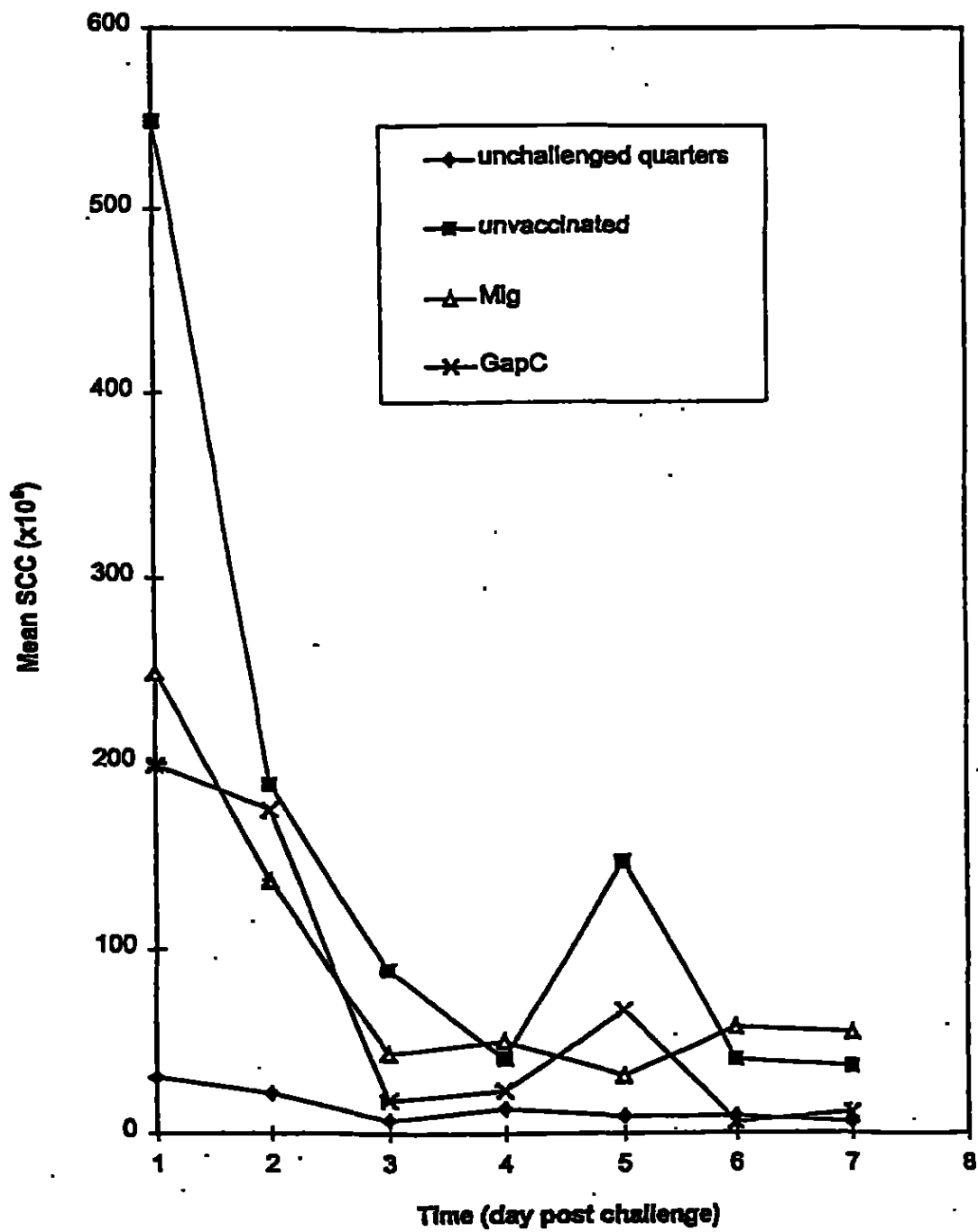


FIG. 5

SUBSTITUTE SHEET (RULE 26)

gca cct aca gtg att gac gca cct gaa cta act oca got ttg act act 672
 Ala Pro Thr Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr
 210 215 220

tac aaa ctt gtt gtt aaa ggt aac act ttc tca gga gaa aca act act 720
 Tyr Lys Leu Val Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr
 225 230 235 240

aaa gcc atc gat act gca act gcg gaa aaa gaa ttc aaa caa tac gca 768
 Lys Ala Ile Asp Thr Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala
 245 250 255

aca gct aac aat gtt gac ggt gag tgg tct tat gac gat gca act aaa 816
 Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys
 260 265 270

acc ttt aca gtt act gaa aaa cca gca gtg att gac gca ctt gaa cta 864
 Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Leu Glu Leu
 275 280 285

act cca gcc ttg act act tac aaa ctt att gtt aaa ggt aac act ttc 912
 Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe
 290 295 300

tca ggc gaa aca act act aaa gct atc gat gct gca act gca gaa aaa 960
 Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp Ala Ala Thr Ala Glu Lys
 305 310 315 320

gaa ttc aaa caa tac gca aca gct aac aat gtt gac ggt gag tgg tct 1008
 Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser
 325 330 335

tat gac tat gca act aaa acc ttt aca gtt act gaa aaa cca gca gtg 1056
 Tyr Asp Tyr Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val
 340 345 350

att gac gca cct gaa cta act cca gcc ttg act act tac aaa ctt att 1104
 Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile
 355 360 365

gtt aaa ggt aac act ttc tca ggc gaa aca act act aaa gct atc gat 1152
 Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp
 370 375 380

gct gca act gca gaa aaa gaa ttc aaa caa tac gca aca gct aac aat 1200
 Ala Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn
 385 390 395 400

gtt gac ggt gaa tgg tct tat gac gat gca act aaa acc ttt aca gtt 1248
 Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val
 405 410 415

FIG. 1B

aag aaa gaa gaa gct aag aaa gct gca act ctt cct aca act ggt gaa 1920
Lys Lys Glu Glu Ala Lys Lys Ala Ala Thr Leu Pro Thr Thr Gly Glu
625 630 635 640

gga agc aac cca ttt ttc aca gct gct gcg ctt gca gta atg gct ggt 1968
Gly Ser Asn Pro Phe Phe Thr Ala Ala Ala Leu Ala Val Met Ala Gly
645 650 655

gcg ggt gct ttg gca gtc gct tca aaa cgt aaa gaa gac taa 2010
Ala Gly Ala Leu Ala Val Ala Ser Lys Arg Lys Glu Asp
660 665 670

FIG. 1D

42

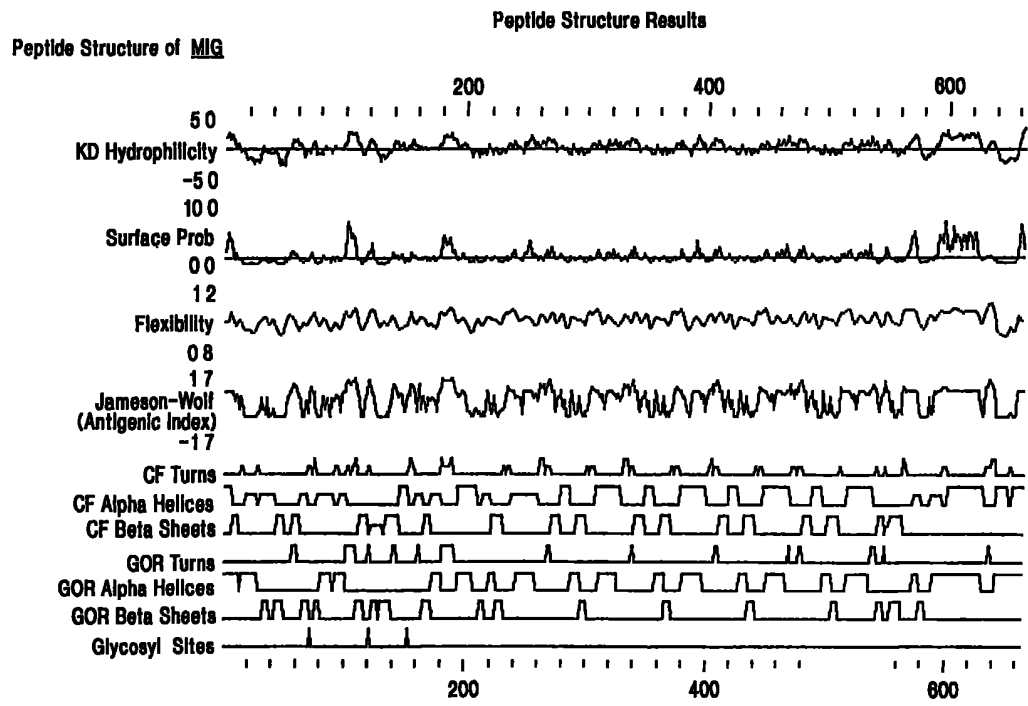


FIG 2

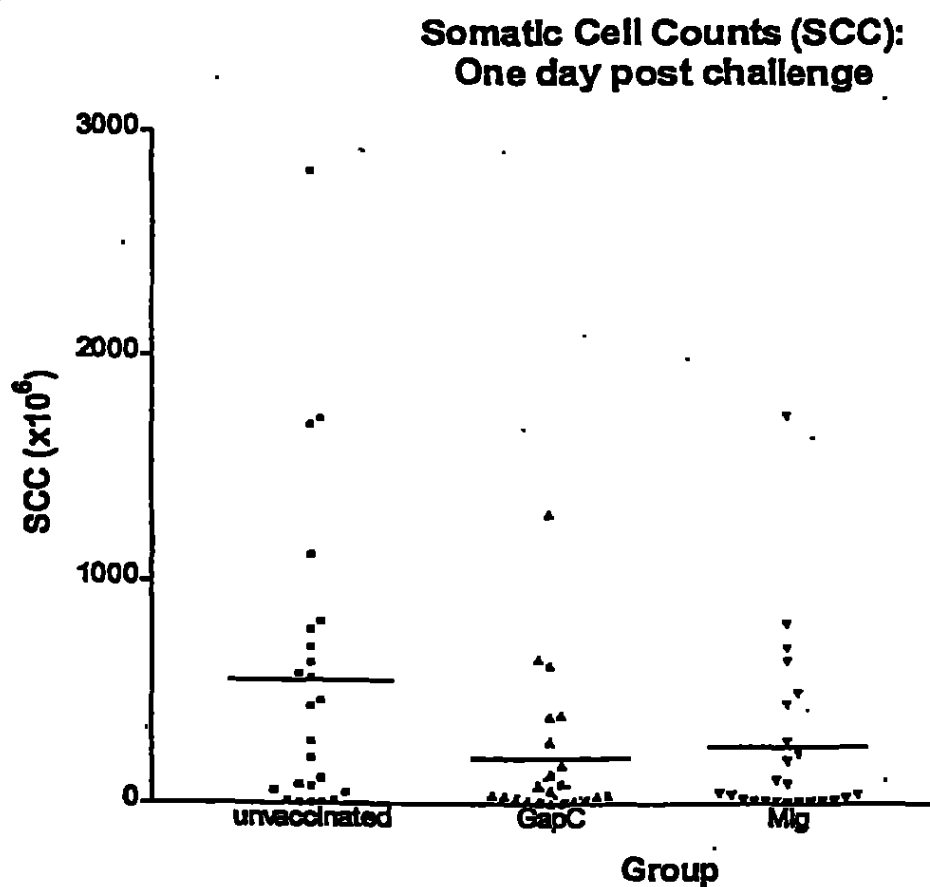


FIG. 6

97
91

SEQUENCE LISTING

<110> University of Saskatchewan
 Potter, Andrew A.
 Bolton, Alexandra J.
 Song, Xin Ming

<120> IMMUNIZATION OF DAIRY CATTLE WITH MIG PROTEIN

<130> 08-891815WO

<140>

<141>

<160> 15

<170> PatentIn Ver. 2.0

<210> 1

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig1

<400> 1

gogggcatgg tagaaaatac tataactg

28

<210> 2

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig1R

<400> 2

acgcccgggt tagtcttctt tacgttt

27

<210> 3

<211> 2010

<212> DNA

<213> Streptococcus dysgalactiae

<220>

<221> CDS

<222> (1) .. (2010)

<400> 3

atg gaa aaa gaa aaa gta aaa tac ttt tta cgt aaa tca gct ttt 48

Met Glu Lys Glu Lys Lys Val Lys Tyr Phe Leu Arg Lys Ser Ala Phe

1

5

10

15

93
92

gga tta gcg tct gta tca gct gcg ttt tta gtt tcg gga gca cta gaa	96
Gly Leu Ala Ser Val Ser Ala Ala Phe Leu Val Ser Gly Ala Leu Glu	
20 25 30	
aat act ata act gtt tct gca gaa act ata cct gca gcg gtc att gta	144
Asn Thr Ile Thr Val Ser Ala Glu Thr Ile Pro Ala Ala Val Ile Val	
35 40 45	
cct gtt ggc cta gat act aca gaa tta caa aaa tgg tat gac att gca	192
Pro Val Gly Leu Asp Thr Thr Glu Leu Gln Lys Trp Tyr Asp Ile Ala	
50 55 60	
aat gat tta gtt gcg act gac aat gct act ccg gga ggc gta ttt aca	240
Asn Asp Leu Val Ala Thr Asp Asn Ala Thr Pro Gly Gly Val Phe Thr	
65 70 75 80	
gca gac tca atg aag gca tta tat cgt tta cta aat gat gca tac gat	288
Ala Asp Ser Met Lys Ala Leu Tyr Arg Leu Leu Asn Asp Ala Tyr Asp	
85 90 95	
gtg ttg gaa tca aaa gac tat aga aaa tat gat tct caa gat agy att	336
Val Leu Glu Ser Lys Asp Tyr Arg Lys Tyr Asp Ser Gln Asp Arg Ile	
100 105 110	
gtt gaa ttg gta aac aat tta aag aat act acg cag tct ctt tta cca	384
Val Glu Leu Val Asn Asn Leu Lys Asn Thr Thr Gln Ser Leu Leu Pro	
115 120 125	
att gga gta gaa cca gta gta ttt gat act act cgc ttg aat acc tgg	432
Ile Gly Val Glu Pro Val Val Phe Asp Thr Thr Arg Leu Asn Thr Trp	
130 135 140	
tat gat gct gct aat gaa att gtt aat aat tca gat gct tat aca gca	480
Tyr Asp Ala Ala Asn Glu Ile Val Asn Asn Ser Asp Ala Tyr Thr Ala	
145 150 155 160	
gaa tca att cag tcg ttg tat aag tta att aat gat gca tac gat gtg	528
Glu Ser Ile Gln Ser Leu Tyr Lys Leu Ile Asn Asp Ala Tyr Asp Val	
165 170 175	
tta gaa tca aaa gat tac agt aag tat gat tct caa gat aaa gtc aac	576
Leu Glu Ser Lys Asp Tyr Ser Lys Tyr Asp Ser Ser Gln Asp Lys Val Asn	
180 185 190	
aat ctt gca gat cag ttg aga gat gca gtt cag gca gtt caa cta gaa	624
Asn Leu Ala Asp Gln Leu Arg Asp Ala Val Gln Ala Val Gln Leu Glu	
195 200 205	
gca cct aca gtg att gac gca cct gaa cta act cca gct ttg act act	672
Ala Pro Thr Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr	
210 215 220	
tac aaa ctt gtt gtt aaa ggt aac act ttc tca gga gaa aca act act	720
Tyr Lys Leu Val Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr	
225 230 235 240	

aaa gcc atc gat act gca act gcg gaa aaa gaa ttc aaa caa tac gca	768
Lys Ala Ile Asp Thr Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala	
245 250 255	
aca gct aac aat gtt gac ggt gag tgg tct tat gac gat gca act aaa	816
Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys	
260 265 270	
acc ttt aca gtt act gaa aaa cca gca gtg att gac gca ctt gaa cta	864
Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Leu Glu Leu	
275 280 285	
act cca gcc ttg act act tac aaa ctt att gtt aaa ggt aac act ttc	912
Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe	
290 295 300	
tca ggc gaa aca act act aaa gct atc gat gct gca act gca gaa aaa	960
Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp Ala Ala Thr Ala Glu Lys	
305 310 315 320	
gaa ttc aaa caa tac gca aca gct aac aat gtt gac ggt gag tgg tct	1008
Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser	
325 330 335	
tat gac tat gca act aaa acc ttt aca gtt act gaa aaa cca gca gtg	1056
Tyr Asp Tyr Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val	
340 345 350	
att gac gca cct gaa cta act cca gcc ttg act act tac aaa ctt att	1104
Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile	
355 360 365	
gtt aaa ggt aac act ttc tca ggc gaa aca act act aaa gct atc gat	1152
Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp	
370 375 380	
gct gca act gca gaa aaa gaa ttc aaa caa tac gca aca gct aac aat	1200
Ala Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn	
385 390 395 400	
gtt gac ggt gaa tgg tct tat gac gat gca act aaa acc ttt aca gtt	1248
Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val	
405 410 415	
act gaa aaa cca gca gtg att gac gca cct gaa cta act cca gcc ttg	1296
Thr Glu Lys Pro Ala Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu	
420 425 430	
act act tac aaa ctt att gtt aaa ggt aac act ttc tca ggc gaa aca	1344
Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe Ser Gly Glu Thr	
435 440 445	
act act aaa gca gta gac gca gaa act gca gaa aaa gcc ttc aaa caa	1392
Thr Thr Lys Ala Val Asp Ala Glu Thr Ala Glu Lys Ala Phe Lys Gln	
450 455 460	

94

tac gca aca gct aac aat gtt gac ggt gaa tgg tct tat gac gat gca	1440
Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala	
465 470 475 480	
act aaa acc ttt aca gtt act gaa aaa cca gca gtg att gac gca cct	1488
Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Pro	
485 490 495	
gaa tta aca cca gca ttg aca acc tac aaa ctt gtt atc aat ggt aaa	1536
Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys	
500 505 510	
aca ttg aaa ggc gaa aca act act aaa gca gta gac gta gaa act gca	1584
Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Val Glu Thr Ala	
515 520 525	
gaa aaa gcc ttc aaa caa tac gct aac gaa aac ggt gtt gat ggt gtt	1632
Glu Lys Ala Phe Lys Gln Tyr Ala Asn Glu Asn Gly Val Asp Gly Val	
530 535 540	
tgg act tac gat gat gcg act aag acc ttt acg gta act gaa atg gtt	1680
Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Met Val	
545 550 555 560	
act gaa att cct ggt gat gca cca act gaa cca gaa aag cca gaa gca	1728
Thr Glu Ile Pro Gly Asp Ala Pro Thr Glu Pro Glu Lys Pro Glu Ala	
565 570 575	
agt atc cct ctt gtt cgg tta act cct gca act cca att gct aaa gat	1776
Ser Ile Pro Leu Val Pro Leu Thr Pro Ala Thr Pro Ile Ala Lys Asp	
580 585 590	
gac gct aag aaa gac gat act aag aaa gtc gat act aag aaa gaa gac	1824
Asp Ala Lys Lys Asp Asp Thr Lys Lys Val Asp Thr Lys Lys Glu Asp	
595 600 605	
gct aaa aaa cca gaa gct aaa aaa cca gaa gct aag aaa gaa gaa gct	1872
Ala Lys Lys Pro Glu Ala Lys Lys Pro Glu Ala Lys Lys Glu Glu Ala	
610 615 620	
aag aaa gaa gaa gct aag aaa gct gca act ctt cct aca act ggt gaa	1920
Lys Lys Glu Glu Ala Lys Lys Ala Ala Thr Leu Pro Thr Thr Gly Glu	
625 630 635 640	
gga agc aac cca ttt ttc aca gct gct gcg ctt gca gta atg gct ggt	1968
Gly Ser Asn Pro Phe Phe Thr Ala Ala Ala Leu Ala Val Met Ala Gly	
645 650 655	
gcg ggt gct ttg gca gtc gct tca aaa cgt aaa gaa gac taa	2010
Ala Gly Ala Leu Ala Val Ala Ser Lys Arg Lys Glu Asp	
660 665 670	

<210> 4
 <211> 669
 <212> PRT

95

7/9

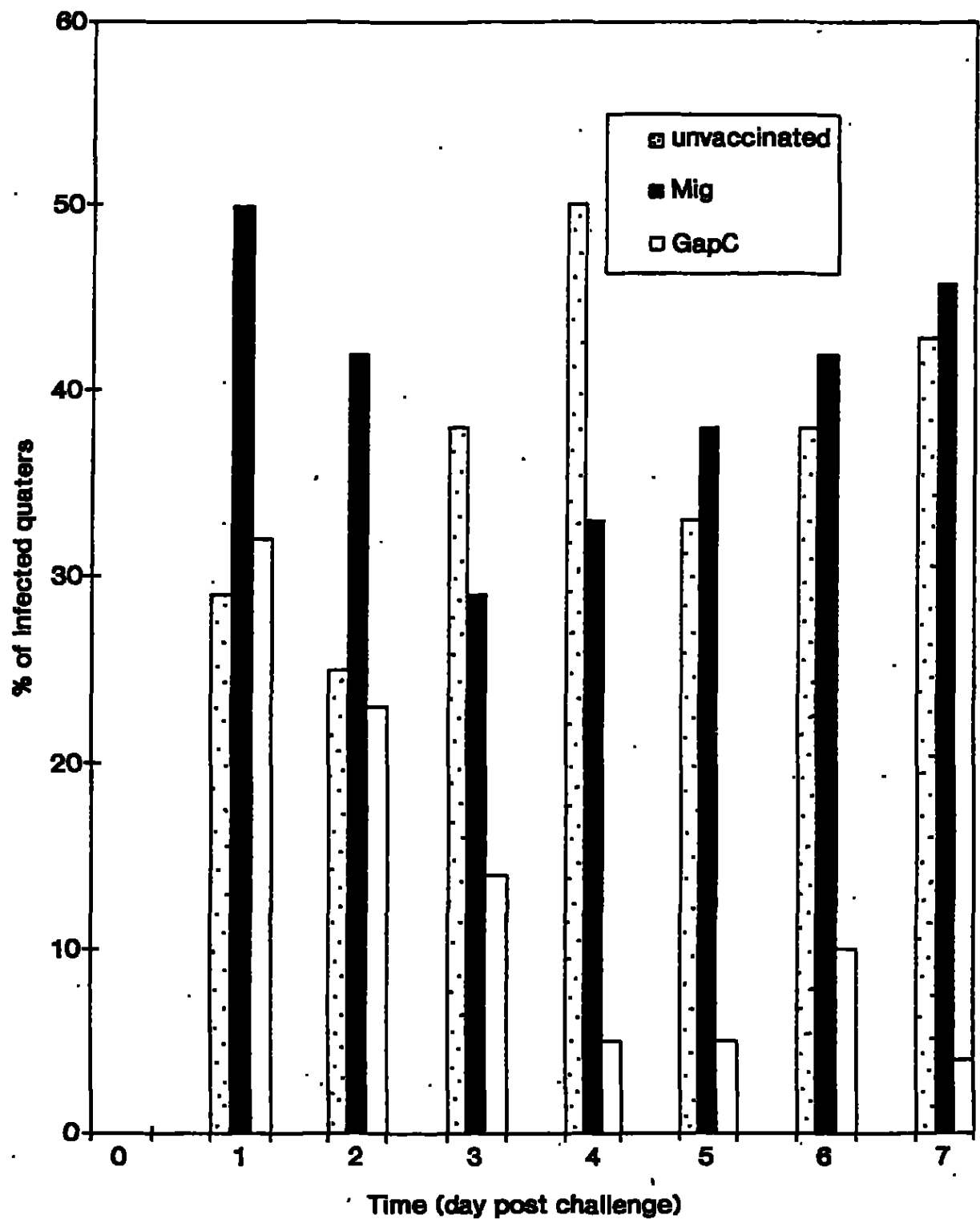
Percentage of quaters infected with *S. dysgalactiae* per group

FIG. 4

SUBSTITUTE SHEET (RULE 26)

96

<213> Streptococcus dysgalactiae

<400> 4

Met Glu Lys Glu Lys Lys Val Lys Tyr Phe Leu Arg Lys Ser Ala Phe
 1 5 10 15

Gly Leu Ala Ser Val Ser Ala Ala Phe Leu Val Ser Gly Ala Leu Glu
 20 25 30

Asn Thr Ile Thr Val Ser Ala Glu Thr Ile Pro Ala Ala Val Ile Val
 35 40 45

Pro Val Gly Leu Asp Thr Thr Glu Leu Gln Lys Trp Tyr Asp Ile Ala
 50 55 60

Asn Asp Leu Val Ala Thr Asp Asn Ala Thr Pro Gly Gly Val Phe Thr
 65 70 75 80

Ala Asp Ser Met Lys Ala Leu Tyr Arg Leu Leu Asn Asp Ala Tyr Asp
 85 90 95

Val Leu Glu Ser Lys Asp Tyr Arg Lys Tyr Asp Ser Gln Asp Arg Ile
 100 105 110

Val Glu Leu Val Asn Asn Leu Lys Asn Thr Thr Gln Ser Leu Leu Pro
 115 120 125

Ile Gly Val Glu Pro Val Val Phe Asp Thr Thr Arg Leu Asn Thr Trp
 130 135 140

Tyr Asp Ala Ala Asn Glu Ile Val Asn Asn Ser Asp Ala Tyr Thr Ala
 145 150 155 160

Glu Ser Ile Gln Ser Leu Tyr Lys Leu Ile Asn Asp Ala Tyr Asp Val
 165 170 175

Leu Glu Ser Lys Asp Tyr Ser Lys Tyr Asp Ser Gln Asp Lys Val Asn
 180 185 190

Asn Leu Ala Asp Gln Leu Arg Asp Ala Val Gln Ala Val Gln Leu Glu
 195 200 205

Ala Pro Thr Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr
 210 215 220

Tyr Lys Leu Val Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr
 225 230 235 240

Lys Ala Ile Asp Thr Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala
 245 250 255

Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys
 260 265 270

Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Leu Glu Leu
 275 280 285

97
47

Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe
 290 295 300
 Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp Ala Ala Thr Ala Glu Lys
 305 310 315 320
 Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser
 325 330 335
 Tyr Asp Tyr Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val
 340 345 350
 Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Ile
 355 360 365
 Val Lys Gly Asn Thr Phe Ser Gly Glu Thr Thr Thr Lys Ala Ile Asp
 370 375 380
 Ala Ala Thr Ala Glu Lys Glu Phe Lys Gln Tyr Ala Thr Ala Asn Asn
 385 390 395 400
 Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val
 405 410 415
 Thr Glu Lys Pro Ala Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu
 420 425 430
 Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe Ser Gly Glu Thr
 435 440 445
 Thr Thr Lys Ala Val Asp Ala Glu Thr Ala Glu Lys Ala Phe Lys Gln
 450 455 460
 Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala
 465 470 475 480
 Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Pro
 485 490 495
 Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys
 500 505 510
 Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Val Glu Thr Ala
 515 520 525
 Glu Lys Ala Phe Lys Gln Tyr Ala Asn Glu Asn Gly Val Asp Gly Val
 530 535 540
 Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Met Val
 545 550 555 560
 Thr Glu Ile Pro Gly Asp Ala Pro Thr Glu Pro Glu Lys Pro Glu Ala
 565 570 575
 Ser Ile Pro Leu Val Pro Leu Thr Pro Ala Thr Pro Ile Ala Lys Asp
 580 585 590

98
98

Asp Ala Lys Lys Asp Asp Thr Lys Lys Val Asp Thr Lys Lys Glu Asp
595 600 605

Ala Lys Lys Pro Glu Ala Lys Lys Pro Glu Ala Lys Lys Glu Glu Ala
610 615 620

Lys Lys Glu Glu Ala Lys Lys Ala Ala Thr Leu Pro Thr Thr Gly Glu
625 630 635 640

Gly Ser Asn Pro Phe Phe Thr Ala Ala Ala Leu Ala Val Met Ala Gly
645 650 655

Ala Gly Ala Leu Ala Val Ala Ser Lys Arg Lys Glu Asp
660 665

<210> 5

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-3

<400> 5

gttggcctag atatcacaga attacaa

27

<210> 6

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-4

<400> 6

aaagcaccog ggccagccat tactg

25

<210> 7

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-6

<400> 7

aggtgcttcc catggaactg cctgaact

28

<210> 8

<211> 30

<212> DNA

<213> Artificial Sequence

94

<220>

<223> Description of Artificial Sequence: primer mig-7

<400> 8

ggcgagagtc tagaaactaa agcgaaaaac

30

<210> 9

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-8

<400> 9

gcaatcacca ggatcctcag taaccatttc

30

<210> 10

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-9

<400> 10

caggcaggtc atatggaagc acctacagt

29

<210> 11

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-10

<400> 11

tcccggagta gcattgtcag tc

22

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer mig-11

<400> 12

gcagcgggcc atatgcctgt tggcctagat

30

<210> 13

<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer mig-12

<400> 13
gcctgaactg gatccctcaa ctgatctg 28

<210> 14
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer mig-13

<400> 14
ttccgttgga tcctgcaact ccaattg 27

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer mig-14

<400> 15
taagtcaaaa gctttgacaa ttagtctt 28

3/9

act gaa aaa cca gca gtg att gac gca cct gaa cta act cca gcc ttg	1296
Thr Glu Lys Pro Ala Val Ile Asp Ala Pro Glu Leu Thr Pro Ala Leu	
420 425 430	
act act tac aaa ctt att gtt aaa ggt aac act ttc tca ggc gaa aca	1344
Thr Thr Tyr Lys Leu Ile Val Lys Gly Asn Thr Phe Ser Gly Glu Thr	
435 440 445	
act act aaa gca gta gac gca gaa act gca gaa aaa gcc ttc aaa caa	1392
Thr Thr Lys Ala Val Asp Ala Glu Thr Ala Glu Lys Ala Phe Lys Gln	
450 455 460	
tac gca aca gct aac aat gtt gac ggt gaa tgg tct tat gac gat gca	1440
Tyr Ala Thr Ala Asn Asn Val Asp Gly Glu Trp Ser Tyr Asp Asp Ala	
465 470 475 480	
act aaa acc ttt aca gtt act gaa aaa cca gca gtg att gac gca cct	1488
Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Ala Val Ile Asp Ala Pro	
485 490 495	
gaa tta aca cca gca ttg aca acc tac aaa ctt gtt atc aat ggt aaa	1536
Glu Leu Thr Pro Ala Leu Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys	
500 505 510	
aca ttg aaa ggc gaa aca act act aaa gca gta gac gta gaa act gca	1584
Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Val Glu Thr Ala	
515 520 525	
gaa aaa gcc ttc aaa caa tac gct aac gaa aac ggt gtt gat ggt gtt	1632
Glu Lys Ala Phe Lys Gln Tyr Ala Asn Glu Asn Gly Val Asp Gly Val	
530 535 540	
tgg act tac gat gat gcg act aag acc ttt acg gta act gaa atg gtt	1680
Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Met Val	
545 550 555 560	
act gaa att cct ggt gat gca cca act gaa cca gaa aag cca gaa gca	1728
Thr Glu Ile Pro Gly Asp Ala Pro Thr Glu Pro Glu Lys Pro Glu Ala	
565 570 575	
agt atc cct ctt gtt ccg tta act cct gca act cca att gct aaa gat	1776
Ser Ile Pro Leu Val Pro Leu Thr Pro Ala Thr Pro Ile Ala Lys Asp	
580 585 590	
gac gct aag aaa gac gat act aag aaa gtc gat act aag aaa gaa gac	1824
Asp Ala Lys Lys Asp Asp Thr Lys Lys Val Asp Thr Lys Lys Glu Asp	
595 600 605	
gct aaa aaa cca gaa gct aaa aaa cca gaa gct aag aaa gaa gaa gct	1872
Ala Lys Lys Pro Glu Ala Lys Lys Pro Glu Ala Lys Lys Glu Glu Ala	
610 615 620	

FIG. 1C

SUBSTITUTE SHEET (RULE 26)



Industria y Comercio

SUPERINTENDENCIA

Radicación : 02112914 00000000
 Fecha (AND): 2002-12-12 16:50:10
 Trámite : 011 PCT-PATENT 379 FASE NNCI 411 PRESENTAC
 Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Folios: 102

C66 13578-B41-001-1

NIT : 800174007-2

RECIBO OFICIAL DE CASH : 02 - 02.545
 FECHA : DICIEMBRE 9 DE 2002

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CARILIER /BOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	3136937	09/12/2002	18,350,000.00

***** C O N C E P T O *****

CANT. IDENTIFIC	CONCEPTO	TOTAL CONCEPTO
1 50005-01 01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	378,000.00
	TOTAL :	378,000.00

SON: TRESCIENTOS SETENTA Y OCHO MIL PESOS

Carrera 13 No. 27-00 Pisos 5, 7 y 10
 Conmutador 382 0840
 Fax 350 5220
 E-mail: info@sic.gov.co
 www.sic.gov.co
 Bogotá, D.C., Colombia

RESPONSABLE : _____

103

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

		USUARIO	RADICADOR
PATENTE DE INVENCION	MODELO DE UTILIDAD	()	(x)
- Indicación de que se solicita la concesión de una patente.		()	(x)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.		()	(x)
- Descripción de la invención.		()	(x)
- Dibujos de ser estos pertinentes.		()	(x)
- Comprobante de Pago de las tasas establecidas.		()	(x)
COMPLETA	(x)	INCOMPLETA	()
DISEÑO INDUSTRIAL	()		
- Indicación de que se solicita el registro de Diseño Industrial		()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.		()	()
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.		()	()
- Comprobante de pago de las tasas establecidas		()	()
COMPLETA	()	INCOMPLETA	()
ESQUEMA DE TRAZADO	()		
- Indicación de que solicita el registro de un Esquema de trazado.		()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.		()	()
- Representación gráfica del esquema de trazado		()	()
- Comprobante de pago de las tasas establecidas.		()	()
COMPLETA	()	INCOMPLETA	()

Firma Responsable:
 Eddy Navarro

12-12-2002

Fecha: