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(54) **Title:** OUTER MEMBRANE VESICLE (OMV) VACCINE COMPRISING N. MENINGITIDIS SEROGROUP B OUTER MEMBRANE PROTEINS

(57) **Abstract:** A composition comprising (a) *Neisseria meningitidis* serogroup B outer membrane vesicles (OMVs), and (b) an immunogenic component selected from other *Neisseria* proteins, or immunogenic fragments thereof. Component (b) preferably includes a protein from a different NmB strain from that from which the OMV of component (a) is derived. The OMVs are preferably obtained by deoxycholate extraction. Optionally, the composition may also comprise a protective antigen against other pathogens.

OUTER MEMBRANE VESICLE (OMV) VACCINE COMPRISING N. MENINGITIDIS SEROGROUP B OUTER MEMBRANE PROTEINS

All documents cited herein are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

This invention relates to vaccines against *Neisseria meningitidis*, serogroup B (NmB).

BACKGROUND ART

Neisseria meningitidis is a non-motile, Gram-negative diplococcus human pathogen. It colonises the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks (see Lieberman *et al.* (1996) *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been identified. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Following the success of the vaccination against *H.influenzae*, however, conjugate vaccines against serogroups A and C have been developed

Serogroup B remains a problem, however, and it is currently responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of α(2-8)-linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if a response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) *Clin Microbiol Rev* 7(4):559-575).

An efficacious outer-membrane vesicle (OMV) vaccine against serogroup B has been produced by the Norwegian National Institute of Public Health [*e.g.* Bjune *et al.* (1991) *Lancet*

338 (8775): 1093-96]. Whilst this vaccine is safe and prevents NmB disease, its efficacy is limited to the strain used to make the vaccine. Other vaccines based around outer-membrane preparations have also been reported. However, there is a clear need in the art for vaccines against NmB that provide protection against several Neisserial strains.

DISCLOSURE OF THE INVENTION

Surprisingly, it has been found that the addition of further defined components to OMV vaccines significantly broadens their efficacy.

Thus the present invention provides a composition comprising (a) a NmB outer membrane preparation, and (b) an immunogenic component selected from one or more of the following:

- a protein disclosed inW099/57280, or an immunogenic fragment thereof;
- a protein disclosed inW099/36544, or an immunogenic fragment thereof;
- a protein disclosed inW099/24578, or an immunogenic fragment thereof;
- a protein disclosed in WO00/66791, or an immunogenic fragment thereof;
- a protein disclosed in Tettelin etal. [Science (2000) 287: 1809-1815], or an immunogenic fragment thereof;
- a protein disclosed in Parkhill etal. [Nature (2000) 404: 502-506], or an immunogenic fragment thereof;
- a protein disclosed in W097/28273, or an immunogenic fragment thereof;
- a protein disclosed inW096/29412, or an immunogenic fragment thereof;
- a protein disclosed in W095/03413, or an immunogenic fragment thereof;
- a protein disclosed inW099/31132, or an immunogenic fragment thereof;
- a protein disclosed inW099/58683, or an immunogenic fragment thereof;
- a protein disclosed in W099/55873, or an immunogenic fragment thereof; and/or
- Neisseria meningitidis protein PorA, TbpA, TbpB,PilC, OpA, or Omp85.

Preferably, the present invention provides a composition comprising (a) outer membrane vesicles from *N.meningitidis* serogroup B; and (b) an immunogenic component comprising (i) an amino acid sequence selected from the group consisting of

MFKRSVIAMACIFPLSACGGGGGGSPDVKSADTPSKPAAPVVAENAGEGVLPKEKKDEEAAGGAPQADTQDATAGEG
SQDMAAVSAENTGNGGAATTDNPKNEDAGAQNDMPQNAAESANQTGNNQPAGSSDSAPASNPAPANGGSDFGRTNV
GNSVVIDGPSQNITLTHCKGDSCNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKDGTNKYIIF

YTDKPPTRSARSRRSLPAEIPLIPVNQADTLIVDGEAVSLTGHSGNIPAPEGNYRYLTYGAEKLPGGSYALRVQGEPAKGEMLVCTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKDRD,MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGTDSSTPNHTPDPNMLAGNMENQATDAGESSQPANQPDMANAADGMQGDDPSAGGQNAGNTAAQGANQAGNNQAAGSSDPIPASNPAPANGGSNFGRVDLANGVLIDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKPKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD,

and

MFKRSVIAMACIVALSACGGGGGGSPDVKSADTLSKPAAPVVTEDVGEEVLPKEKKDEEAVSGAPQADTQDATAGKGGQDMAAVSAENTGNGGAATTDNPENKDEGPQNDMPQNAADTDSSTPNHTPAPNMPTRDMGNQAPDAGESAQPANQPDMANAADGMQGDDPSAGENAGNTADQAANQAENNQVGGSQNPASSTNPNATNGGSDFGRINVANGIKLDSGSENVTLTHCKDKVCDRDFLDEEAPPKSEFEKLSDEEKINKYKKDEQRENFVGLVADRVEKNGTNKYVIIYKDKSASSSSARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAVIDGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD, (ii) a fragment of 10 or more contiguous amino acids of (i), and/or (iii) an amino acid sequence having 70% or more sequence identity to (i).

If the composition comprises a protein disclosed in W099/24578, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, & 892, as disclosed in WO99/24578 (or a protein comprising an immunogenic fragment of one or more of these SEQ IDs, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these SEQ IDs).

If the composition comprises a protein disclosed in WO99/36544, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, & 90, as disclosed in WO99/36544 (or a protein comprising an immunogenic fragment of one or more of these SEQ IDs, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these SEQ IDs).

If the composition comprises a protein disclosed in Tettelin *et al.* (*i.e.* a protein encoded by one of the genes disclosed therein), said protein preferably comprises an amino acid sequence selected from the group consisting of NMB0001 to NMB2160 (or a protein comprising an immunogenic fragment of one or more of these 2160 genes, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these 2160 genes).

If the composition comprises a protein disclosed in Parkhill *et al.*, said protein preferably comprises an amino acid sequence selected from the group consisting of the 2121 coding sequences disclosed therein (or a protein comprising an immunogenic fragment of one or more of these 2121 sequences, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these 2121 sequences).

If the composition comprises a protein disclosed in WO99/57280, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 898, 900, 902, 904, 906, 908, 910, 912, 914, 916, 918, 920, 922, 924, 926, 928, 930, 932, 934, 936, 938, 940, 942, 944, 946, 948, 950, 952, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1068, 1070, 1072, 1074, 1076, 1078, 1080, 1082, 1084, 1086, 1088, 1090, 1092, 1094, 1096, 1098, 1100, 1102, 1104, 1106, 1108, 1110, 1112, 1114, 1116, 1118, 1120, 1122, 1124, 1126, 1128, 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1462, 1464, 1466, 1468, 1470, 1472, 1474, 1476, 1478, 1480, 1482, 1484, 1486, 1488, 1490, 1492, 1494, 1496, 1498, 1500, 1502, 1504, 1506, 1508, 1510, 1512, 1514, 1516, 1518, 1520, 1522, 1524, 1526, 1528, 1530, 1532, 1534, 1536, 1538, 1540, 1542, 1544, 1546, 1548, 1550, 1552, 1554, 1556, 1558, 1560, 1562, 1564, 1566, 1568, 1570, 1572, 1574, 1576, 1578, 1580, 1582, 1584, 1586, 1588, 1590, 1592, 1594, 1596, 1598, 1600, 1602, 1604, 1606, 1608, 1610, 1612, 1614, 1616, 1618, 1620, 1622, 1624, 1626, 1628, 1630, 1632, 1634, 1636, 1638, 1640, 1642, 1644, 1646, 1648, 1650, 1652, 1654, 1656, 1658, 1660, 1662, 1664, 1666, 1668, 1670, 1672, 1674, 1676, 1678, 1680, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1706, 1708, 1710, 1712, 1714, 1716, 1718, 1720, 1722, 1724, 1726, 1728, 1730, 1732, 1734, 1736, 1738, 1740, 1742, 1744, 1746, 1748, 1750, 1752, 1754, 1756, 1758, 1760, 1762, 1764, 1766, 1768, 1770, 1772, 1774, 1776, 1778, 1780, 1782, 1784, 1786, 1788, 1790, 1792, 1794, 1796, 1798, 1800, 1802, 1804, 1806, 1808, 1810, 1812, 1814, 1816, 1818, 1820, 1822, 1824, 1826, 1828, 1830, 1832, 1834, 1836, 1838, 1840, 1842, 1844, 1846, 1848, 1850, 1852, 1854, 1856, 1858, 1860, 1862, 1864, 1866, 1868, 1870, 1872, 1874, 1876, 1878, 1880, 1882, 1884, 1886, 1888, 1890, 1892, 1894, 1896, 1898, 1900, 1902, 1904, 1906, 1908, 1910, 1912, 1914, 1916, 1918, 1920, 1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, 2082, 2084, 2086, 2088, 2090, 2092, 2094, 2096, 2098, 2100, 2102, 2104, 2106, 2108, 2110, 2112, 2114, 2116, 2118, 2120, 2122, 2124, 2126, 2128, 2130, 2132, 2134, 2136, 2138, 2140, 2142, 2144, 2146, 2148, 2150, 2152, 2154, 2156, 2158, 2160, 2162, 2164, 2166, 2168, 2170, 2172, 2174, 2176, 2178, 2180, 2182, 2184, 2186, 2188, 2190, 2192, 2194, 2196, 2198, 2200, 2202, 2204, 2206, 2208, 2210, 2212, 2214, 2216, 2218, 2220, 2222, 2224, 2226, 2228, 2230, 2232, 2234, 2236, 2238, 2240, 2242, 2244, 2246, 2248, 2250, 2252, 2254, 2256, 2258, 2260, 2262, 2264, 2266, 2268, 2270, 2272, 2274, 2276, 2278, 2280, 2282, 2284, 2286, 2288, 2290, 2292, 2294, 2296, 2298, 2300, 2302, 2304, 2306, 2308, 2310, 2312, 2314, 2316, 2318, 2320, 2322, 2324, 2326, 2328, 2330, 2332, 2334, 2336, 2338, 2340, 2342, 2344, 2346, 2348, 2350, 2352, 2354, 2356, 2358, 2360, 2362, 2364, 2366, 2368, 2370, 2372, 2374, 2376, 2378, 2380, 2382, 2384, 2386, 2388, 2390, 2392, 2394, 2396, 2398, 2400, 2402, 2404, 2406, 2408, 2410, 2412, 2414, 2416, 2418, 2420, 2422, 2424, 2426, 2428, 2430, 2432, 2434, 2436, 2438, 2440, 2442, 2444, 2446, 2448, 2450, 2452, 2454, 2456, 2458, 2460, 2462, 2464, 2466, 2468, 2470, 2472, 2474, 2476, 2478, 2480, 2482, 2484, 2486, 2488, 2490, 2492, 2494, 2496, 2498, 2500, 2502, 2504, 2506, 2508, 2510, 2512, 2514, 2516, 2518, 2520, 2522, 2524, 2526, 2528, 2530, 2532, 2534, 2536, 2538, 2540, 2542, 2544, 2546, 2548, 2550, 2552, 2554, 2556, 2558, 2560, 2562, 2564, 2566, 2568, 2570, 2572, 2574, 2576, 2578, 2580, 2582, 2584, 2586, 2588, 2590, 2592, 2594, 2596, 2598, 2600, 2602, 2604, 2606, 2608, 2610, 2612, 2614, 2616, 2618, 2620, 2622, 2624, 2626, 2628, 2630, 2632, 2634, 2636, 2638, 2640, 2642, 2644, 2646, 2648, 2650, 2652, 2654, 2656, 2658, 2660, 2662, 2664, 2666, 2668, 2670, 2672, 2674, 2676, 2678, 2680, 2682, 2684, 2686, 2688, 2690, 2692, 2694, 2696, 2698, 2700, 2702, 2704, 2706, 2708, 2710, 2712, 2714, 2716, 2718, 2720, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2764, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2782, 2784, 2786, 2788, 2790, 2792, 2794, 2796, 2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, 2836, 2838, 2840, 2842, 2844, 2846, 2848, 2850, 2852, 2854, 2856, 2858, 2860, 2862, 2864, 2866, 2868, 2870, 2872, 2874, 2876, 2878, 2880, 2882, 2884, 2886, 2888, 2890, 2892, 2894, 2896, 2898, 2900, 2902, 2904, 2906, 2908, 2910, 2912, 2914, 2916, 2918, 2920, 2922, 2924, 2926, 2928, 2930, 2932, 2934, 2936, 2938, 2940, 2942, 2944, 2946, 2948, 2950, 2952, 2954, 2956, 2958, 2960, 2962, 2964, 2966, 2968, 2970, 2972, 2974, 2976, 2978, 2980, 2982, 2984, 2986, 2988, 2990, 2992, 2994, 2996, 2998, 3000, 3002, 3004, 3006, 3008, 3010, 3012, 3014, 3016, 3018 & 3020, as disclosed in WO99/57280 (or a protein comprising an immunogenic fragment of one or more of these SEQ IDs, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these SEQ IDs).

If the composition comprises a protein disclosed in WO99/28273, said protein is preferably the protein disclosed in Figure 4 or Figure 13 of WO97/28273.

If the composition comprises a protein disclosed in WO96/29412, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ IDs 1-8 disclosed in WO96/29412 (or a protein comprising an immunogenic fragment of one or more of these SEQ IDs, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these SEQ IDs).

If the composition comprises a protein disclosed in WO95/03413, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ IDs 1-23 disclosed in WO95/03413 (or a protein comprising an immunogenic fragment of one or more of these SEQ IDs, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to one of these SEQ IDs).

If the composition comprises a protein disclosed in WO99/31132, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ ID 2 disclosed in WO99/31132 (or a protein comprising an immunogenic fragment of SEQ ID 2, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 2).

If the composition comprises a protein disclosed in WO99/58683, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ ID 2 or SEQ ID 4 disclosed in WO99/58683 (or a protein comprising an immunogenic fragment of SEQ ID 2 or SEQ ID 4, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 2 or SEQ ID 4).

If the composition comprises a protein disclosed in WO99/55873, said protein preferably comprises an amino acid sequence selected from the group consisting of SEQ ID 2 or SEQ ID 4 disclosed in WO99/55873 (or a protein comprising an immunogenic fragment of SEQ ID 2 or SEQ ID 4, or a protein comprising a sequence having sequence identity (preferably greater than 50% *eg*. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 2 or SEQ ID 4).

Details of Opa and PorA can be found in Wiertz *et al.* [*Infect. Immun.* (1996) 61:298-304]. PilC is disclosed in Nassif *et al.* [*PNAS USA* (1994) 91:3769-73]. Omp85 is disclosed in Manning *et al.* [*Microb. Pathog.* (1998) 25:11-21]. TbpA and TbpB are disclosed in Ala'Aldeen & Borriello [*Vaccine* (1996) 14:49-53] and also in Legrain *et al.* [*Prorein Expr Purif* (1995) 6:570-578].

Preferred proteins for component (b) are:

- protein '919', typified by SEQ IDs 3069-3074 and 3207-3241 of WO99/57280 (see also Figure 23 and Example 15 therein).
- protein '235', typified by SEQ IDs 869-874 and 3149-3178 of WO99/57280 (see also Figure 20 and Example 12 therein).
- protein '519', typified by SEQ IDs 3045-3056 and 3185-3206 of WO99/57280 (see also Figure 22 and Example 14 therein).
- protein '225', typified by SEQ IDs 793-804 and 3115-3148 of WO99/57280 (see also Figure 19 and Example 11 therein).
- protein 'ORF40', typified by example 1 (SEQ IDs 1-6) of WO99/36544 (see also Figure 1 of WO00/66741; see also WO99/31132 and WO99/58683).
- protein '287', typified by example 9 of WO99/57280 (see SEQ IDs 1199-1204, 3103-3108 and 3179-3184 therein).
- protein 'ORF1', typified by example 77 (SEQ IDs 647-654) of WO99/24578 (see also WO99/55873 and accession number AJ242535).
- protein 'ORF4', typified by example 26 (SEQ IDs 215-226) of WO99/24578 (see also Figure 2 of WO00/66741).
- protein 'ORF46', typified by example 55 (SEQ IDs 457-466) of WO99/24578 (see also Figure 12 of WO00/66741).

Component (b) of the composition is preferably a NmB protein. It is preferred that component (b) includes a protein from a different NmB strain from that from which the OMV of component (a) is derived *i.e.* the OMV in component (a) is preferably supplemented by immunogenic component (b) from a different NmB strain.

One or more of the components (or all of them) may be adsorbed on $Al(OH)_3$.

The outer membrane preparation component

The compositions of the invention include a NmB outer membrane preparation as component (a). This is preferably in the form of outer membrane vesicles (OMVs).

The preparation of OMVs from NmB is well-known in the art. Methods for obtaining suitable preparations are disclosed in, for instance: Claassen *et al.* [*Vaccine* (1996) 14:1001-1008]; Cartwright *et al.* [*Vaccine* (1999) 17:2612-2619]; Peeters *et al.* [*Vaccine* (1996) 14:1009-

1015]; Fu *et al.* [*Biotechnology NY* (1995) 12:170-74]; Davies *et al.* [*J.Immunol.Meth.* (1990) 134:215-225]; Saunders *et al.* [*Infect. Immun.* (1999) 67:113-119]; Draabick *et al.* [*Vaccine* (2000) 18:160-172]; Moreno *et al.* [*Infect. Immun.* (1985) 47:527-533]; Milagres *et al.* [*Infect. Immun.* (1994) 62:4419-4424]; Naess *et al.* [*Infect. Immun.* (1998) 66:959-965]; Rosenqvist *et al.* [*Dev.Biol.Stand.* (1998) 92:323-333]; Haneberg *et al.* [*Infect. Immunn.* (1998) 66:1334-41]; Andersen *et al.* [*Vaccine* (1997) 15:1225-34]; Bjune *et al.* [*Lancet* (1991) 338:1093-96] *etc.*

OMVs are preferably a deoxycholate extract from NmB (*i.e.* obtained from NmB by deoxycholate extraction). The preferred extraction protocol is that described by Fredriksen *et al.* [Production, characterization and control of MenB-vaccine "Folkehelsa": an outer membrane vesicle vaccine against group B meningococcal disease (1991) *NIPH Ann.* 14(2):67-80].

A preferred strain from which to extract OMVs is the 44/76 strain (B:15:P1.7,16:P5.5:L3,7,9) of *N.meningitidis*.

Further details of the OMV component can be found in, for instance, Bjune *et al.* [*Lancet* (1991) 338(8775):1093-96], or Fredriksen *et al.* [Characterization of high molecular weight component in MenB-vaccine 'Folkehelsa', an outer membrane vesicle vaccine against group B meningococcal disease. Pages 818-824 of *Pathobiology and immunobiology of Neisseriaceae* (*eds.* Conde-Glez *et al.*) ISBN 968-6502-13-0].

The OMV component may be adsorbed to aluminium hydroxide adjuvant. A preferred protein:adjuvant ratio is 1:67 (wt/wt).

A typical dose of vaccine for a human contains 25μg protein, 2μg LPS and 1.67mg $Al(OH)_3$, and can be injected in 0.5ml volumes into the deltoid muscle.

The OMV component (*e.g.* as obtained by deoxycholate extraction) may be treated to remove certain components. For instance, pyrogens or toxic components may be removed (*e.g.* LPS).

It is preferred that the OMV component should retain the 80kDa antigenic component described by Fredriksen *et al.* [pages 818-824 of *Pathobiology and immunobiology of Neisseriaceae*].

More preferably, the OMV component should retain a protein comprising one or more of the following amino acid sequences: SEQ ID 3, SEQ ID 5, SEQ ID 7, SEQ ID 9, SEQ ID 11, SEQ ID 13 [or (i) a protein having sequence identity to SEQ ID 3, SEQ ID 5, SEQ ID 7, SEQ ID 9, SEQ ID 11, or SEQ ID 13 – depending on the particular SEQ ID, the degree of sequence identity is preferably greater than 50% (*eg*. 60%, 70%, 80%, 90%, 95%, 99% or more), which includes mutants and allelic variants, or (ii) a protein comprising an immunogenic fragment of SEQ ID 1, SEQ ID 3, SEQ ID 5, SEQ ID 7, SEQ ID 9, SEQ ID 11, or SEQ ID 13 – the fragment should comprise at least *n* consecutive amino acids from the sequence and, depending on the particular sequence, *n* is 7 or more (*eg*. 8, 10, 12, 14, 16, 18, 20 or more).]

Combining components (a) and (b)

Components (a) and (b) can be combined by simply mixing component (a) with an outer-membrane preparation (*e.g.* by mixing ORF4 with Norwegian OMVs).

As an alternative, they can be combined by manipulating a bacterium such that it produces (preferably hyperproduces) component (a) in its outer membrane – an outer-membrane preparation from such a recombinant bacterium will comprise both component (a) and component (b).

Suitable bacteria for manipulation in this way include *Neisseria meningitidis* (any serogroup or strain), *Neisseria lactamica, Neisseria cinerea* or any other non-typable *Neisseria*. Other Gram-negative bacteria can also be used, such as *E.coli, Salmonella, Shigella, Bordetella, Yersinia, Helicobacter, etc.* Transformation methods are well known in the art.

Multivalent vaccines

Optionally, the composition of the invention may also comprise one or more of the following components:

- a protective antigen against *Neisseria meningitidis* serogroup A;
- a protective antigen against *Neisseria meningitidis* serogroup C;
- a protective antigen against *Neisseria meningitidis* serogroup Y;
- a protective antigen against *Neisseria meningitidis* serogroup W;
- a protective antigen against *Haemophilus influenzae*;
- a protective antigen against *pneumococcus*;
- a protective antigen against diphtheria;
- a protective antigen against tetanus;
- a protective antigen against whooping cough;

- a protective antigen against *Helicobacter pylori*;
- a protective antigen against polio; and/or
- a protective antigen against hepatitis B virus.

Preferred examples of these optional components are:

- a polysaccharide antigen against *Neisseria meningitidis* serogroup A;
- a polysaccharide antigen against *Neisseria meningitidis* serogroup C, such as that described in Costantino *et al.* (1992) *Vaccine* 10:691-698;
- a polysaccharide antigen against *Neisseria meningitidis* serogroup Y;
- a polysaccharide antigen against *Neisseria meningitidis* serogroup W;
- a polysaccharide antigen against *Haemophilus influenzae*;
- a polysaccharide antigen against *pneumococcus*;
- a protective antigen against diphtheria, consisting of a diphtheria toxoid, such as the CRM197 mutant [*eg.* Del Guidice *et al.* (1998) *Molecular Aspects of Medicine* 19:1-70].
- a protective antigen against tetanus, consisting of a tetanus toxoid [*eg.* Wassilak & Orenstein, Chapter 4 of *Vaccines* (eds. Plotkin & Mortimer), 1988]
- a protective antigen against whooping cough, comprising pertussis holotoxin (PT) and filamentous haemagglutinin (FHA); optionally further comprising pertactin and/or agglutinogens 2 and 3 [*eg.* Gustafsson *et al.* (1996) *N. Engl. J. Med.* 334:349-355; Rappuoli *et al.* (1991) *TIBTECH* 9:232-238].
- a protective antigen against *H.pylori*, comprising one or more of CagA (*eg.* WO93/18150), VacA (*eg.* WO93/18150), NAP (*eg.* WO99/53310), HopX (*eg.* WO98/04702), HopY (*eg.* WO98/04702), urease.
- a protective antigen against hepatitis B virus, consisting of a HBV surface antigen and/or a HBV core antigen.

Where the composition comprises an antigen against diphtheria, it preferably also comprises antigens against tetanus and polio. Where the composition comprises an antigen against tetanus, it preferably also comprises antigens against diphtheria and polio. Where the composition comprises an antigen against polio, it preferably also comprises antigens against diphtheria and tetanus.

Pertussis toxin is a toxic protein and, when present in the composition, it is preferably detoxified. Detoxification may be by chemical and/or genetic means. A preferred detoxified mutant is the 9K/129G double mutant [*eg*. Rappuoli (1997) *Nature Medicine* 3:374-376].

Where the composition includes a protein that exists in different nascent and mature forms, the mature form of the protein is preferably used. For example, where NspA is included, (WO96/29412; see also Martin *et al.* (1997) *J. Exp. Med* 185 1173-1183) the mature form of the protein lacking the signal peptide is preferably used.

Where the composition includes a polysaccharide antigen, the polysaccharide is preferably conjugated to a carrier protein.

Therapy, prophylaxis, diagnosis

The composition of the invention is preferably a vaccine. Vaccines according to the invention may either be prophylactic (*ie.* to prevent infection) or therapeutic (*ie.* to treat disease after infection).

The invention also provides the compositions of the invention for use as medicaments (preferably as vaccines) or as diagnostic reagents. It also provides the use of a composition according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria. Said Neisserial bacteria may be any species or strain (such as *N.gonorrhoeae*) but are preferably *N.meningitidis*, especially serogroup B (NmB).

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to the invention. The method is preferably immunisation.

Processes

According to further aspects, the invention provides various processes.

A process for producing a composition of the invention is provided, comprising the step of extraction (*e.g.* deoxycholate extraction) of OMVs from *N.meningitidis*.

SEQUENCE LISTING

The sequences in the sequence listing are:

SEQ ID	DESCRIPTION
1	N-terminal sequence of *N.meningitidis* serogroup B protein, 80-85kDa
2	Complete gene from *N.meningitidis* serogroup B
3	Encoded protein from SEQ ID 2
4	Signal peptide protein from SEQ ID 3
5	Mature protein from SEQ ID 3
6	Complete gene from *N.gonorrhoeae*, homologous to SEQ ID 2
7	Encoded protein from SEQ ID 6
8	Signal peptide protein from SEQ ID 7
9	Mature protein from SEQ ID 7
10	Complete gene from *N.meningitidis* serogroup A, homologous to SEQ ID 2
11	Encoded protein from SEQ ID 10
12	Signal peptide protein from SEQ ID 11
13	Mature protein from SEQ ID 11
14	Protein '919' from nmb strain 2996

MODES FOR CARRYING OUT THE INVENTION

A summary of standard techniques and procedures which may be employed in order to perform the invention (*eg.* to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *eg.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and*

Practice, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

Proteins used with the invention can be prepared by various means (*eg*. recombinant expression, purification from cell culture, chemical synthesis *etc*.) and in various forms (*eg*. native, fusions *etc*.). They are preferably prepared in substantially pure form (*ie*. substantially free from other Neisseria or host cell proteins).

Nucleic acid used with the invention can be prepared in many ways (*eg*. by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc*.) and can take various forms (*eg*. single stranded, double stranded, vectors, probes *etc*.). The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc*.

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" *eg*. a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters gap open penalty=12 and gap extension penalty=1. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (*eg*. see US patent 5,753,235).

Expression systems

The Neisserial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg*. structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual, 2nd ed.*].

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallotheionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive cells.

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science 236*:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J. 4*:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) *Proc. Natl. Acad. Sci. 79*:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell 41*:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet. 2*:215; Maniatis et al. (1987) *Science* 236:1237].

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus triparite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell 41*:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci. 14*:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminater/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg*. plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell 23*:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol. 6*:1074].

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*eg*. Hep G2), and a number of other cell lines.

ii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*eg*. plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) *17*:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, *42*:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli.*

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (*eg*. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* *69*:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, *73*:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α-interferon, Maeda et al., (1985), *Nature 315*:592; human gastrin-releasing peptide, Lebacq-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, *82*:8404; mouse IL-3, (Miyajima et al., (1987) *Gene 58*:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) *3*:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays 4*:91.The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti* , *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol. 56*:153; Wright (1986) *Nature 321*:718; Smith et al., (1983) *Mol. Cell. Biol. 3*:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol. 25*:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See, eg.* Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, eg. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, eg. proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., Nucleic

Acids Research 15:2515-2535 (1987); Wirsel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for Agrobacterium transformations, T DNA sequences for Agrobacterium-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Reptr*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg*. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud *et al.* (1984) *Annu. Rev. Genet.* *18*:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature 198*:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res. 8*:4057; Yelverton *et al.* (1981) *Nucl. Acids Res. 9*:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature 292*:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene 25*:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci. 80*:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol. 189*:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci. 82*:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature 254*:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature 309*:810]. Fusion proteins can also be made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene 60*:197], *trpE* [Allen et al. (1987) J. Biotechnol. 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol. 135*:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology 7*:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb *et al.* (1984) *EMBO J. 3*:2437] and the *E. coli* alkaline phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci. 82*:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various Bacillus strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various Bacillus strains integrate into the Bacillus chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol.* *32*:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: Bacillus subtilis [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* *79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], Escherichia coli [Shimatake *et al.* (1981) *Nature* *292*:128; Amann *et al.* (1985) *Gene* *40*:183; Studier *et al.* (1986) *J. Mol. Biol.* *189*:113; EP-A-0 036 776,EP-A-0 136 829 and EP-A-0 136 907], Streptococcus cremoris [Powell *et al.* (1988)

Appl. Environ. Microbiol. *54*:655]; Streptococcus lividans [Powell *et al.* (1988) *Appl. Environ. Microbiol.* *54*:655], Streptomyces lividans [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *eg.* [Masson *et al.* (1989) *FEMS Microbiol. Lett.* *60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* *79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* *85*:856; Wang *et al.* (1990) *J. Bacteriol.* *172*:949, Campylobacter], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* *69*:2110; Dower *et al.* (1988) *Nucleic Acids Res.* *16*:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* *53*:159; Taketo (1988) *Biochim. Biophys. Acta* *949*:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett.* *44*:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem* *170*:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett.* *66*:203, Staphylococcus], [Barany *et al.* (1980) *J. Bacteriol.* *144*:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* *32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* *54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* *1*:412, Streptococcus].

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:1].

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA 77*:1078; Henikoff *et al.* (1981) *Nature 283*:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol. 96*:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast Saccharomyces cerevisiae," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene 11*:163; Panthier *et al.* (1980) *Curr. Genet. 2*:109;].

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *eg*. EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg*. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*eg*. WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (US patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*eg*. see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg*. plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al*. (1979) *Gene 8*:17-24], pCl/1 [Brake *et al*. (1984) *Proc. Natl. Acad. Sci USA 81*:4642-4646], and YRp17 [Stinchcomb *et al*. (1982) *J. Mol. Biol. 158*:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *eg*. Brake *et al*., *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in Enzymol. 101*:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol, Rev. 51*:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts:Candida albicans [Kurtz, *et al.* (1986) *Mol. Cell. Biol. 6*:142], Candida maltosa [Kunze, *et al.* (1985) *J. Basic Microbiol. 25*:141]. Hansenula polymorpha [Gleeson, *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302], Kluyveromyces fragilis [Das, *et al.* (1984) *J. Bacteriol. 158*:1165], Kluyveromyces lactis [De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:737; Van den Berg *et al.* (1990) *Bio/Technology 8*:135], Pichia guillerimondii [Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141], Pichia pastoris [Cregg, *et al.* (1985) *Mol. Cell. Biol. 5*:3376; US Patent Nos. 4,837,148 and 4,929,555], Saccharomyces cerevisiae [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*:1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163], Schizosaccharomyces pombe [Beach and Nurse (1981) *Nature 300*:706], and Yarrowia lipolytica [Davidow, *et al.* (1985) *Curr. Genet. 10*:380471 Gaillardin, *et al.* (1985) *Curr. Genet. 10*:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *eg.* [Kurtz *et al.* (1986) *Mol. Cell. Biol. 6*:142; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; Candida]; [Gleeson *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302; Hansenula];

[Das *et al.* (1984) *J. Bacteriol. 158*:1165; De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:1165; Van den Berg *et al.* (1990) *Bio/Technology 8*:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol. 5*:3376; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; US Patent Nos. 4,837,148 and 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*;1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163 Saccharomyces]; [Beach and Nurse (1981) *Nature 300*:706; Schizosaccharomyces]; [Davidow *et al.* (1985) *Curr. Genet. 10*:39; Gaillardin *et al.* (1985) *Curr. Genet. 10*:49; Yarrowia].

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying Neisserial proteins.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*eg.* 1,000*g* for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [*Nature* (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*eg*. hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (*eg*. in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions 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. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, *etc.* pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (*eg.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*eg.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The immunogenic compositions (*eg.* the immunising antigen/immunogen/polypeptide/protein/ nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the immunogenic compositions 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 also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg*. nonhuman primate, primate, *etc*.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, *eg*. by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (*eg*. WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [*eg*. Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al*. (1997) *Annu Rev Immunol* 15:617-648; see later herein].

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura

(1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses *eg.* MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*eg.* HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591.624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993)

Cancer Res 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995,WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *eg.* WO93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A.Polypeptides

One example are polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B.Hormones, Vitamins, *etc.*

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C.Polyalkylenes, Polysaccharides, *etc.*

Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethlylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D.Lipids, and Liposomes

The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416);

mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *eg*. Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See *eg*. Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77); Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E.Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) Annu Rev. Biochem 54:699; Law (1986) Adv. Exp Med. Biol. 151:162; Chen (1986) J Biol Chem 261:12918; Kane (1980) Proc Natl Acad Sci USA 77:2465; and Utermann (1984) Hum Genet 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (*supra*); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* WO98/06437.

F.Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic aid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and purtrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

Immunodiagnostic Assays

Neisserial antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-Neisserial antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to Neisserial proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated Tm of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1μg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/μg. For a single-copy mammalian gene a conservative approach would start with 10 μg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/μg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (Tm) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$Tm = 81 + 16.6(\log_{10}Ci) + 0.4[\%(G + C)] - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch}).$$

where Ci is the salt concentration (monovalent ions) and *n* is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Identification of the meningococcal 80-85kDa protein

It was observed that various outer membrane vesicle preparations from *N.meningitidis* serogroup B contained a component of approximately 80-85kDa. This protein was purified from SDS-PAGE gels and N-terminal sequenced (SEQ ID 1).

Antibodies raised against the SDS-PAGE purified protein cross-reacted with equivalent proteins in more than 50 *N.meningitidis* strains of diverse serogroups and serotypes. Cross-reactivity with *N.gonorrhoeae*, *N.polysaccharia* and *N.lactamica* was also observed. Post-immune sera from vaccinated patients also reacted with the protein.

The complete gene was cloned from serogroup B *N.meningitidis* (SEQ ID 2) and the encoded protein was inferred (SEQ ID 3). By comparison with the N-terminal sequencing described above, a signal peptide (SEQ ID 4) and a mature sequence (SEQ ID 5) are inferred.

***Identification of corresponding genes in* N.meningitidis *serogroup A and* N.gonorrhoeae**

On the basis of the serogroup B *N.meningitidis* sequence, the corresponding genes from *N.meningitidis* serogroup A and *N.gonorrhoeae* were cloned and sequenced.

The complete gene from serogroup A *N.meningitidis* is shown in SEQ ID 6, with the encoded protein in SEQ ID 7. The signal peptide and mature sequence are SEQ IDs 8 and 9.

The complete gene from *N.gonorrhoeae* is shown in SEQ ID 10, with the encoded protein in SEQ ID 11. The signal peptide and mature sequence are SEQ IDs 12 and 13.

Sequence comparisons

The protein sequences were compared and are highly homologous.

The *N.meningitidis* serogroup B sequence and the *N.gonorrhoeae* sequence show 95.4% identity in 797 aa overlap:

```
                       10        20        30        40        50        60
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                       70        80        90       100       110       120
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                      190       200       210       220       230       240
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orf21ng.pep    FEGNQVYSDRKLMRQMSLTEGGIWTWLTRSDRFDRQKFAQDMEKVTDFYQNNGYFDFRIL
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orf21ng.pep    DTDIQTNEDKTRQTIKITVHEGGRFRWGKVSIEGDTNEVPKAELEKLLTMKPGKWYERQQ
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orf21ng.pep    MTAVLGEIQNRMGSAGYAYSEISVQPLPNAGTKTVDFVLHIEPGRKIYVNEIHITGNNKT
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orf21ng.pep    RDEVVRRELRQMESAPYDTSKLQRSKERVELLGYFDNVQFDAVPLAGTPDKVDLNMSLTE
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                      430       440       450       460       470       480
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orf21ng.pep    DGVSLGYDIYGKAFDPRKASTSVKQYKTTTAGGGVRMGIPVTEYDRVNFGLAAEHLTVNT
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                      730       740       750       760       770       780
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The *N.meningitidis* serogroup A and B sequences show 99.9% identity in 797 aa overlap:

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                       70        80        90       100       110       120
orf21.pep      IIKSLYATGFFDDVRVETADGQLLLTVIERPTIGSLNITGAKMLQNDAIKKNLESFGLAQ
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orf21a.pep     IIKSLYATGFFDDVRVETADGQLLLTVIERPTIGSLNITGAKMLQNDAIKKNLESFGLAQ
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                      130       140       150       160       170       180
orf21.pep      SQYFNQATLNQAVAGLKEEYLGRGKLNIQITPKVTKLARNRVDIDITIDEGKSAKITDIE
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orf21a.pep     SQYFNQATLNQAVAGLKEEYLGRGKLNIQITPKVTKLARNRVDIDITIDEGKSAKITDIE
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                      190       200       210       220       230       240
orf21.pep      FEGNQVYSDRKLMRQMSLTEGGIWTWLTRSNQFNEQKFAQDMEKVTDFYQNNGYFDFRIL
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orf21a.pep     FEGNQVYSDRKLMRQMSLTEGGIWTWLTRSNQFNEQKFAQDMEKVTDFYQNNGYFDFRIL
                      190       200       210       220       230       240

                      250       260       270       280       290       300
orf21.pep      DTDIQTNEDKTKQTIKITVHEGGRFRWGKVSIEGDTNEVPKAELEKLLTMKPGKWYERQQ
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orf21a.pep     DTDIQTNEDKTKQTIKITVHEGGRFRWGKVSIEGDTNEVPKAELEKLLTMKPGKWYERQQ
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                      310       320       330       340       350       360
orf21.pep      MTAVLGEIQNRMGSAGYAYSEISVQPLPNAETKTVDFVLHIEPGRKIYVNEIHITGNNKT
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orf21a.pep     MTAVLGEIQNRMGSAGYAYSEISVQPLPNAETKTVDFVLHIEPGRKIYVNEIHITGNNKT
                      310       320       330       340       350       360

                      370       380       390       400       410       420
orf21.pep      RDEVVRRELRQMESAPYDTSKLQRSKERVELLGYFDNVQFDAVPLAGTPDKVDLNMSLTE
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orf21a.pep     RDEVVRRELRQMESAPYDTSKLQRSKERVELLGYFDNVQFDAVPLAGTPDKVDLNMSLTE
                      370       380       390       400       410       420
```

```
                   430       440       450       460       470       480
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orf21a.pep  RSTGSLDLSAGWVQDTGLVMSAGVSQDNLFGTGKSAALRASRSKTTLNGSLSFTDPYFTA
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                   490       500       510       520       530       540
orf21.pep   DGVSLGYDVYGKAFDPRKASTSIKQYKTTTAGAGIRMSVPVTEYDRVNFGLVAEHLTVNT
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orf21a.pep  DGVSLGYDVYGKAFDPRKASTSIKQYKTTTAGAGIRMSVPVTEYDRVNFGLVAEHLTVNT
                   490       500       510       520       530       540

                   550       560       570       580       590       600
orf21.pep   YNKAPKHYADFIKKYGKTDGTDGSFKGWLYKGTVGWGRNKTDSALWPTRGYLTGVNAEIA
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orf21a.pep  YNKAPKHYADFIKKYGKTDGTDGSFKGWLYKGTVGWGRNKTDSALWPTRGYLTGVNAEIA
                   550       560       570       580       590       600

                   610       620       630       640       650       660
orf21.pep   LPGSKLQYYSATHNQTWFFPLSKTFTLMLGGEVGIAGGYGRTKEIPFFENFYGGGLGSVR
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf21a.pep  LPGSKLQYYSATHNQTWFFPLSKTFTLMLGGEVGIAGGYGRTKEIPFFENFYGGGLGSVR
                   610       620       630       640       650       660

                   670       680       690       700       710       720
orf21.pep   GYESGTLGPKVYDEYGEKISYGGNKKANVSAELLFPMPGAKDARTVRLSLFADAGSVWDG
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orf21a.pep  GYESGTLGPKVYDEYGEKISYGGNKKANVSAELLFPMPGAKDARTVRLSLFADAGSVWDG
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                   730       740       750       760       770       780
orf21.pep   KTYDDNSSSATGGRVQNIYGAGNTHKSTFTNELRYSAGGAVTWLSPLGPMKFSYAYPLKK
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orf21a.pep  KTYDDNSSSATGGRVQNIYGAGNTHKSTFTNELRYSAGGAVTWLSPLGPMKFSYAYPLKK
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                   790
orf21.pep   KPEDEIQRFQFLGTTF
            ||||||||||||||||
orf21a.pep  KPEDEIQRFQFLGTTFX
                   790
```

The high degree of conservation suggests that a single protein may be able to induce immune responses against a variety of *Neisseriae* species.

Vaccines

The three proteins identified above were expressed and used for immunisation. Good immune responses were observed against the proteins.

Combination vaccines

In addition, the proteins were each combined with antigens against other pathogenic organisms (*e.g.* the Chiron polysaccharide vaccine against serogroup C meningitis). and used for immunisation. Good immune responses were observed.

Further NmB components

Whilst it is efficacious, the protection elicited by the Norwegian OMV vaccine is restricted to the strain used to make the vaccine. The clinical trials on the vaccine obtained only 57.2% efficacy after 29 months in teenagers, although IgG responses were observed in almost 100% of patients [*e.g.* Rosenqvist *et al.* (1995) *Infect. Immun.* 63:4642-4652].

Surprisingly, it has been found that the addition of further defined components to the Norwegian OMV vaccine significantly broadens its efficacy.

The Norwegian vaccine does not elicit protection against NmB strain 2996. Defined proteins from strain 2996 were added to the Norwegian vaccine, and it was shown that the efficacy of the vaccine was increased by a surprising degree. Furthermore, the addition of a NmC polysaccharide conjugate antigen [*e.g.* Costantino *et al.* (1992) *Vaccine* 10:691-698] gave excellent results.

The bactericidal activities of the combinations are shown in the following table:

Group	**Norwegian OMV**	**NmB antigen***	**NmC antigen**	**Bactericidal activity against NmB strain 2996**
1	+	-	-	<4
2	+	#1	-	512
3	+	#2	-	>2048
4	+	#3	-	1024
5	+	#3	+	256
6	-	#3	-	2048
7	-	#3	+	2048

* Three different NmB antigens were used:

#1: ORF1 – *e.g.* example 77 of WO99/24578 (see also WO99/55873)
#2: protein '287' – *e.g.* Figure 21 of WO99/57280 (also SEQ IDs 3103-3108)
#3: a mixture in $Al(OH)_3$ of #1, #2 and protein '919' (SEQ ID 14 herein; see also WO99/57280 Figure 23 and SEQ IDs 3069-3074 therein).

It can readily be seen that the inefficacy of the Norwegian OMV vaccine against strain 2996 (group 1) can be overcome by adding defined antigens from strain 2996. The results using NmB protein '287' are particularly good. The Norwegian vaccine can thus be improved without needing to prepare OMVs from a number of different strains.

This vaccine also offers protection against heterologous MenB strains. The same vaccines, prepared using 2996 strain proteins, was tested against five other strains. Titres were as follows:

Group	2996	BZ133	BZ232	1000	MC58	NGH38
1	<4	1024	<4	>2048	>2048	32
2	512	512	<4	>2048	>2048	256
3	4096	4096	256	1024	>2048	1024
4	1024	2048	<4	2048	>2048	64
5	256	>32000	<4	>2048	>2048	128
6	2048	2048	4	<4	64	4
7	2048	>32000	4	128	1024	128
Control *	32768	4096	8192	16384	16384	8192

* Controls: strains 2996, BZ133 & 1000 = OMVs prepared from homologous strain; strain BZ232 = OMVs prepared from 2996; MC58 & NGH38 = SEAM3

A second study supplemented 'Norwegian' OMVs with proteins from NmB strain 2996:

- protein 919, expressed in *E.coli* without any fusion partner
- ORF1, expressed in *E.coli* as a His-tagged fusion
- Protein 287, expressed in *E.coli* as a GST fusion
- A mixture of these three proteins, optionally with the NmC conjugate

. The preparations were adjuvanted with $Al(OH)_3$ and tested against the homologous strain using the bactericidal assay. Results were as follows:

	NmB			NmC	
Antigen	**2996**	**NGH38**	**394/98**	**C11**	**BZ133**
OMVs	<4	32	1024 *	<4	1024
+ 919	<4	<4	4	<4	512
+ORF1	512	256	2048	4096	512
+287	4096	1024	1024	512	4096
+mix	1024	64	4	64	2048
+mix +NmC	256	128	2048	64000	>32000

* the antibodies were bacteriostatic, not bactericidal

Further work with antigen 287

Combinations of Norwegian OMVs with antigen 287 were investigated further. 20μg antigen 287 was combined with Norwegian OMP vaccine (15μg OMP + $Al(OH)_3$) and used to immunise mice. The antibodies were tested in the bactericidal assay, and were effective against all strains tested. The results were as follows:

	NmB							NmA	NmC
Antigen	**2996**	**BZ133**	**BZ232**	**1000**	**MC58**	**NGH38**	**NZ**	**F6124**	**C11**
OMVs	<4	1024	<4	>2048	32768	32	<4	-	-
287	8000	4096	256	<4	512	2048	1024	1024	2048
OMV+287	4096	4096	256	1024	4096	1024	1024	-	-

In almost all cases, therefore, the combination of OMVs + protein 287 surprisingly gives better results that the OMVs alone.

Recombinant OMVs

E.coli were transformed to express ORF1, ORF40 and ORF46. OMVs prepared from the recombinant *E.coli* were able to induce bactericidal antibodies against *N.meningitidis*.

ORF1, ORF40 and ORF46 (strain 2996) were expressed as His-tagged fusions in *E.coli* and were prepared either as pure proteins or in the form of OMVs. Bactericidal titres against both preparations were tested using strain 2996 as challenge:

Antigen:	**ORF1**	**ORF40**	**ORF46**
Purified	64	2048	16000
OMV	1024	256	128000

Bactericidal titres using heterologous challenge strains were also measured. ORF46 gives a titre against strain MC58 of 4096 in pure form, but this rises to 32000 when in the form of OMVs. ORF1 gives a titre against NmA strain F6124 of 128 in pure form, but this rises to 512 when in the form of OMVs. ORF40 gives a titre against strain MC58 of 512 in pure form, but this doubles when in the form of OMVs.

These data show that NmB antigens retain immunogenicity when prepared in *E.coli* as OMVs and, furthermore, that immunogenicity can actually be enhanced.

It will be understood that this application describes the invention by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

EDITORIAL NOTE

APPLICATION NUMBER – 28754/01

The following Sequence Listing pages 1-21 are part of the description. The claims pages follow on pages 54-56.

SEQUENCE LISTING

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Lys Leu Asn Ile Gln Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn
145                 150                 155                 160

Arg Val Asp Ile Asp Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile
                165                 170                 175

Thr Asp Ile Glu Phe Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu
            180                 185                 190

Met Arg Gln Met Ser Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr
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        195                 200                 205

Arg Ser Asn Gln Phe Asn Glu Gln Lys Phe Ala Gln Asp Met Glu Lys
    210                 215                 220

Val Thr Asp Phe Tyr Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu
225                 230                 235                 240

Asp Thr Asp Ile Gln Thr Asn Glu Asp Lys Thr Lys Gln Thr Ile Lys
                245                 250                 255

Ile Thr Val His Glu Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile
            260                 265                 270

Glu Gly Asp Thr Asn Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu
        275                 280                 285

Thr Met Lys Pro Gly Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val
    290                 295                 300

Leu Gly Glu Ile Gln Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser
305                 310                 315                 320

Glu Ile Ser Val Gln Pro Leu Pro Asn Ala Glu Thr Lys Thr Val Asp
                325                 330                 335

Phe Val Leu His Ile Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile
            340                 345                 350

His Ile Thr Gly Asn Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu
        355                 360                 365

Leu Arg Gln Met Glu Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg
    370                 375                 380

Ser Lys Glu Arg Val Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe
385                 390                 395                 400

Asp Ala Val Pro Leu Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met
                405                 410                 415

Ser Leu Thr Glu Arg Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp
            420                 425                 430

Val Gln Asp Thr Gly Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn
        435                 440                 445

Leu Phe Gly Thr Gly Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys
    450                 455                 460

Thr Thr Leu Asn Gly Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala
465                 470                 475                 480

Asp Gly Val Ser Leu Gly Tyr Asp Val Tyr Gly Lys Ala Phe Asp Pro
                485                 490                 495

Arg Lys Ala Ser Thr Ser Ile Lys Gln Tyr Lys Thr Thr Thr Ala Gly
            500                 505                 510

Ala Gly Ile Arg Met Ser Val Pro Val Thr Glu Tyr Asp Arg Val Asn
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        515                 520                 525

Phe Gly Leu Val Ala Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala
    530                 535                 540

Pro Lys His Tyr Ala Asp Phe Ile Lys Lys Tyr Gly Lys Thr Asp Gly
545                 550                 555                 560

Thr Asp Gly Ser Phe Lys Gly Trp Leu Tyr Lys Gly Thr Val Gly Trp
                565                 570                 575

Gly Arg Asn Lys Thr Asp Ser Ala Leu Trp Pro Thr Arg Gly Tyr Leu
            580                 585                 590

Thr Gly Val Asn Ala Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr
        595                 600                 605

Tyr Ser Ala Thr His Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr
    610                 615                 620

Phe Thr Leu Met Leu Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly
625                 630                 635                 640

Arg Thr Lys Glu Ile Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu
                645                 650                 655

Gly Ser Val Arg Gly Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr
            660                 665                 670

Asp Glu Tyr Gly Glu Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn
        675                 680                 685

Val Ser Ala Glu Leu Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg
    690                 695                 700

Thr Val Arg Leu Ser Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly
705                 710                 715                 720

Lys Thr Tyr Asp Asp Asn Ser Ser Ser Ala Thr Gly Gly Arg Val Gln
                725                 730                 735

Asn Ile Tyr Gly Ala Gly Asn Thr His Lys Ser Thr Phe Thr Asn Glu
            740                 745                 750

Leu Arg Tyr Ser Ala Gly Gly Ala Val Thr Trp Leu Ser Pro Leu Gly
        755                 760                 765

Pro Met Lys Phe Ser Tyr Ala Tyr Pro Leu Lys Lys Lys Pro Glu Asp
    770                 775                 780

Glu Ile Gln Arg Phe Gln Phe Gln Leu Gly Thr Thr Phe
785                 790                 795

<210>  4
<211>  21
<212>  PRT
<213>  Neisseria meningitidis

<400>  4
Met Lys Leu Lys Gln Ile Ala Ser Ala Leu Met Met Leu Gly Ile Ser
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1               5                   10                  15

Pro Leu Ala Leu Ala
                20

<210>   5
<211>   776
<212>   PRT
<213>   Neisseria meningitidis

<400>   5
Asp Phe Thr Ile Gln Asp Ile Arg Val Glu Gly Leu Gln Arg Thr Glu
1               5                   10                  15

Pro Ser Thr Val Phe Asn Tyr Leu Pro Val Lys Val Gly Asp Thr Tyr
            20                  25                  30

Asn Asp Thr His Gly Ser Ala Ile Ile Lys Ser Leu Tyr Ala Thr Gly
        35                  40                  45

Phe Phe Asp Asp Val Arg Val Glu Thr Ala Asp Gly Gln Leu Leu Leu
    50                  55                  60

Thr Val Ile Glu Arg Pro Thr Ile Gly Ser Leu Asn Ile Thr Gly Ala
65                  70                  75                  80

Lys Met Leu Gln Asn Asp Ala Ile Lys Lys Asn Leu Glu Ser Phe Gly
                85                  90                  95

Leu Ala Gln Ser Gln Tyr Phe Asn Gln Ala Thr Leu Asn Gln Ala Val
            100                 105                 110

Ala Gly Leu Lys Glu Glu Tyr Leu Gly Arg Gly Lys Leu Asn Ile Gln
        115                 120                 125

Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn Arg Val Asp Ile Asp
    130                 135                 140

Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile Thr Asp Ile Glu Phe
145                 150                 155                 160

Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu Met Arg Gln Met Ser
                165                 170                 175

Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr Arg Ser Asn Gln Phe
            180                 185                 190

Asn Glu Gln Lys Phe Ala Gln Asp Met Glu Lys Val Thr Asp Phe Tyr
        195                 200                 205

Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu Asp Thr Asp Ile Gln
    210                 215                 220

Thr Asn Glu Asp Lys Thr Lys Gln Thr Ile Lys Ile Thr Val His Glu
225                 230                 235                 240

Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile Glu Gly Asp Thr Asn
                245                 250                 255

Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu Thr Met Lys Pro Gly
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            260                 265                 270

Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val Leu Gly Glu Ile Gln
        275                 280                 285

Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser Glu Ile Ser Val Gln
    290                 295                 300

Pro Leu Pro Asn Ala Glu Thr Lys Thr Val Asp Phe Val Leu His Ile
305                 310                 315                 320

Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile His Ile Thr Gly Asn
                325                 330                 335

Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu Leu Arg Gln Met Glu
            340                 345                 350

Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg Ser Lys Glu Arg Val
        355                 360                 365

Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe Asp Ala Val Pro Leu
    370                 375                 380

Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met Ser Leu Thr Glu Arg
385                 390                 395                 400

Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp Val Gln Asp Thr Gly
                405                 410                 415

Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn Leu Phe Gly Thr Gly
            420                 425                 430

Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys Thr Thr Leu Asn Gly
        435                 440                 445

Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala Asp Gly Val Ser Leu
    450                 455                 460

Gly Tyr Asp Val Tyr Gly Lys Ala Phe Asp Pro Arg Lys Ala Ser Thr
465                 470                 475                 480

Ser Ile Lys Gln Tyr Lys Thr Thr Thr Ala Gly Ala Gly Ile Arg Met
                485                 490                 495

Ser Val Pro Val Thr Glu Tyr Asp Arg Val Asn Phe Gly Leu Val Ala
            500                 505                 510

Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala Pro Lys His Tyr Ala
        515                 520                 525

Asp Phe Ile Lys Lys Tyr Gly Lys Thr Asp Gly Thr Asp Gly Ser Phe
    530                 535                 540

Lys Gly Trp Leu Tyr Lys Gly Thr Val Gly Trp Gly Arg Asn Lys Thr
545                 550                 555                 560

Asp Ser Ala Leu Trp Pro Thr Arg Gly Tyr Leu Thr Gly Val Asn Ala
                565                 570                 575

Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr Tyr Ser Ala Thr His
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            580                 585                 590

Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr Phe Thr Leu Met Leu
        595                 600                 605

Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly Arg Thr Lys Glu Ile
    610                 615                 620

Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu Gly Ser Val Arg Gly
625                 630                 635                 640

Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr Asp Glu Tyr Gly Glu
                645                 650                 655

Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn Val Ser Ala Glu Leu
            660                 665                 670

Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg Thr Val Arg Leu Ser
        675                 680                 685

Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly Lys Thr Tyr Asp Asp
    690                 695                 700

Asn Ser Ser Ser Ala Thr Gly Gly Arg Val Gln Asn Ile Tyr Gly Ala
705                 710                 715                 720

Gly Asn Thr His Lys Ser Thr Phe Thr Asn Glu Leu Arg Tyr Ser Ala
                725                 730                 735

Gly Gly Ala Val Thr Trp Leu Ser Pro Leu Gly Pro Met Lys Phe Ser
            740                 745                 750

Tyr Ala Tyr Pro Leu Lys Lys Lys Pro Glu Asp Glu Ile Gln Arg Phe
        755                 760                 765

Gln Phe Gln Leu Gly Thr Thr Phe
    770                 775

<210>   6
<211>   2379
<212>   DNA
<213>   Neisseria gonorrhoeae

<400>   6
atgaaactga aacagattgc ctccgcactg atgatgttgg gcatatcgcc tttggcattt     60
gccgacttca ccatccaaga catccgtgtc gaaggcttgc agcgtaccga gccgagcacc    120
gtattcaact acctgcccgt caaagtcggc gacacctaca acgacacaca cggcagtgcc    180
atcatcaaaa gcctgtacgc caccggtttc tttgacgacg tacgagtcga aactgcggac    240
gggcagcttc tgctgaccgt tatcgaacgc cccaccatcg gctcgctcaa catcaccggc    300
gccaaaatgc tgcaaaacga cgccatcaag aaaaacctcg aatcgttcgg gctggcgcag    360
tcgcaatact ttaatcaggc gacactcaac caggcagtcg ccggcctgaa agaagaatac    420
ctcgggcgtg gcaaactcaa tatccaaatc acgcccaaag taaccaaact cgcccgcaac    480
cgcgtcgaca tcgacatcac gattgacgag ggcaaatccg ccaaaatcac cgacatcgaa    540
tttgaaggca accaagtcta ttccgaccgc aaactgatgc ggcagatgtc gctgaccgaa    600
ggcggcattt ggacatggct gacacgaagc gaccggttcg accgccagaa attcgcccaa    660
gacatggaaa aagtaaccga cttctaccag aacaacggct acttcgattt ccgtatcctc    720
gataccgaca tccaaaccaa cgaagacaaa accaggcaga ccatcaaaat caccgtccac    780
gaaggcggac gtttccgctg gggcaaagtg tcgattgaag gcgacaccaa cgaagtcccc    840
aaggccgaac tggaaaaact gctgaccatg aagcccggca aatggtacga acgccagcag    900
atgaccgccg ttttgggtga gattcagaac cgcatgggct cggcaggcta cgcatacagc    960
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gaaatcagcg tacagccgct gccgaacgcc ggaaccaaaa ccgtcgattt cgtcctgcac   1020
atcgaaccgg gccggaaaat ctacgtcaac gaaatccaca tcaccggcaa caacaaaacc   1080
cgcgacgaag tcgtgcgccg cgaattgcgc caaatggaat ccgcgcctta cgacacctcc   1140
aagctgcaac gctccaaaga gcgcgtcgag cttttgggct acttcgacaa cgtacagttt   1200
gatgccgtcc cgcttgccgg tacgcccgac aaagtcgatt tgaacatgag cctgaccgaa   1260
cgctccaccg gctcgctcga cttgagcgcg ggctgggttc aggataccgg cttggtcatg   1320
tccgccggcg tatcgcagga caacctgttc ggtacgggca agtcggccgc cctgcgcgcc   1380
tcgcgaagca aaaccacgct caacggctcg ctgtcgttta ccgacccgta cttcacggca   1440
gacggggtca gcctgggcta cgatatttac ggaaaagcct tcgacccgcg caaagcatcg   1500
accagcgtca aacaatataa aaccaccacc gccggcggcg gcgtaaggat gggtatcccc   1560
gttaccgaat acgaccgcgt caatttcggg ctggcggcgg aacacctgac cgtcaacacc   1620
tacaacaaag cacccaaacg ctatgccgac tttatcagga aatacggcaa aaccgacggc   1680
gcagacggca gcttcaaagg cctgctgtac aaaggcaccg tcggctgggg gcgcaacaag   1740
accgacagcg cgtcatggcc gacgcgcggc tacctgaccg gcgtaaatgc cgaaatcgcc   1800
ctgcccggca gcaaactgca atactactcc gccacccaca accaaacctg gttcttcccc   1860
ttaagcaaaa ccttcacgct gatgctcggc ggcgaagtcg gcattgcggg cggctacggc   1920
agaaccaaag aaatcccctt ctttgaaaac ttctacggcg gcggcctggg ttcggtgcgc   1980
ggctacgaaa gcggcacgct cggcccgaaa gtgtatgacg aatacggcga aaaaatcagc   2040
tacggcggca acaaaaaagc caacgtctcc gccgagctgc tcttcccgat gcccggtgcg   2100
aaagacgcac gcaccgtccg cctgagcctg tttgccgacg caggcagcgt gtgggacggc   2160
agaacctata ccgccgccga aaacggtaac aacaaatcgg tttactcgga aaacgcgcat   2220
aaatccacct ttaccaacga attgcgctat tccgccggcg gcgcggttac ctggctctcg   2280
cctttgggtc cgatgaaatt cagctacgcc tacccgctga agaaaaaacc ggaagacgaa   2340
atccaacgct tccaattcca gctcggcacg acgttctaa                          2379

<210>  7
<211>  792
<212>  PRT
<213>  Neisseria gonorrhoeae

<400>  7
Met Lys Leu Lys Gln Ile Ala Ser Ala Leu Met Met Leu Gly Ile Ser
1               5                   10                  15

Pro Leu Ala Phe Ala Asp Phe Thr Ile Gln Asp Ile Arg Val Glu Gly
            20                  25                  30

Leu Gln Arg Thr Glu Pro Ser Thr Val Phe Asn Tyr Leu Pro Val Lys
        35                  40                  45

Val Gly Asp Thr Tyr Asn Asp Thr His Gly Ser Ala Ile Ile Lys Ser
    50                  55                  60

Leu Tyr Ala Thr Gly Phe Phe Asp Asp Val Arg Val Glu Thr Ala Asp
65                  70                  75                  80

Gly Gln Leu Leu Leu Thr Val Ile Glu Arg Pro Thr Ile Gly Ser Leu
                85                  90                  95

Asn Ile Thr Gly Ala Lys Met Leu Gln Asn Asp Ala Ile Lys Lys Asn
            100                 105                 110

Leu Glu Ser Phe Gly Leu Ala Gln Ser Gln Tyr Phe Asn Gln Ala Thr
        115                 120                 125

Leu Asn Gln Ala Val Ala Gly Leu Lys Glu Glu Tyr Leu Gly Arg Gly
    130                 135                 140

Lys Leu Asn Ile Gln Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn
145                 150                 155                 160
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Arg Val Asp Ile Asp Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile
                165                 170                 175

Thr Asp Ile Glu Phe Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu
            180                 185                 190

Met Arg Gln Met Ser Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr
        195                 200                 205

Arg Ser Asp Arg Phe Asp Arg Gln Lys Phe Ala Gln Asp Met Glu Lys
    210                 215                 220

Val Thr Asp Phe Tyr Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu
225                 230                 235                 240

Asp Thr Asp Ile Gln Thr Asn Glu Asp Lys Thr Arg Gln Thr Ile Lys
                245                 250                 255

Ile Thr Val His Glu Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile
            260                 265                 270

Glu Gly Asp Thr Asn Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu
        275                 280                 285

Thr Met Lys Pro Gly Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val
    290                 295                 300

Leu Gly Glu Ile Gln Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser
305                 310                 315                 320

Glu Ile Ser Val Gln Pro Leu Pro Asn Ala Gly Thr Lys Thr Val Asp
                325                 330                 335

Phe Val Leu His Ile Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile
            340                 345                 350

His Ile Thr Gly Asn Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu
        355                 360                 365

Leu Arg Gln Met Glu Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg
    370                 375                 380

Ser Lys Glu Arg Val Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe
385                 390                 395                 400

Asp Ala Val Pro Leu Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met
                405                 410                 415

Ser Leu Thr Glu Arg Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp
            420                 425                 430

Val Gln Asp Thr Gly Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn
        435                 440                 445

Leu Phe Gly Thr Gly Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys
    450                 455                 460

Thr Thr Leu Asn Gly Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala
465                 470                 475                 480
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```
Asp Gly Val Ser Leu Gly Tyr Asp Ile Tyr Gly Lys Ala Phe Asp Pro
                485                 490                 495

Arg Lys Ala Ser Thr Ser Val Lys Gln Tyr Lys Thr Thr Thr Ala Gly
            500                 505                 510

Gly Gly Val Arg Met Gly Ile Pro Val Thr Glu Tyr Asp Arg Val Asn
        515                 520                 525

Phe Gly Leu Ala Ala Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala
    530                 535                 540

Pro Lys Arg Tyr Ala Asp Phe Ile Arg Lys Tyr Gly Lys Thr Asp Gly
545                 550                 555                 560

Ala Asp Gly Ser Phe Lys Gly Leu Leu Tyr Lys Gly Thr Val Gly Trp
                565                 570                 575

Gly Arg Asn Lys Thr Asp Ser Ala Ser Trp Pro Thr Arg Gly Tyr Leu
            580                 585                 590

Thr Gly Val Asn Ala Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr
        595                 600                 605

Tyr Ser Ala Thr His Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr
    610                 615                 620

Phe Thr Leu Met Leu Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly
625                 630                 635                 640

Arg Thr Lys Glu Ile Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu
                645                 650                 655

Gly Ser Val Arg Gly Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr
            660                 665                 670

Asp Glu Tyr Gly Glu Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn
        675                 680                 685

Val Ser Ala Glu Leu Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg
    690                 695                 700

Thr Val Arg Leu Ser Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly
705                 710                 715                 720

Arg Thr Tyr Thr Ala Ala Glu Asn Gly Asn Asn Lys Ser Val Tyr Ser
                725                 730                 735

Glu Asn Ala His Lys Ser Thr Phe Thr Asn Glu Leu Arg Tyr Ser Ala
            740                 745                 750

Gly Gly Ala Val Thr Trp Leu Ser Pro Leu Gly Pro Met Lys Phe Ser
        755                 760                 765

Tyr Ala Tyr Pro Leu Lys Lys Lys Pro Glu Asp Glu Ile Gln Arg Phe
    770                 775                 780

Gln Phe Gln Leu Gly Thr Thr Phe
785                 790
```

```
<210>   8
<211>   21
<212>   PRT
<213>   Neisseria gonorrhoeae

<400>   8
Met Lys Leu Lys Gln Ile Ala Ser Ala Leu Met Met Leu Gly Ile Ser
1               5                   10                  15

Pro Leu Ala Phe Ala
            20

<210>   9
<211>   771
<212>   PRT
<213>   Neisseria gonorrhoeae

<400>   9
Asp Phe Thr Ile Gln Asp Ile Arg Val Glu Gly Leu Gln Arg Thr Glu
1               5                   10                  15

Pro Ser Thr Val Phe Asn Tyr Leu Pro Val Lys Val Gly Asp Thr Tyr
            20                  25                  30

Asn Asp Thr His Gly Ser Ala Ile Ile Lys Ser Leu Tyr Ala Thr Gly
        35                  40                  45

Phe Phe Asp Asp Val Arg Val Glu Thr Ala Asp Gly Gln Leu Leu Leu
    50                  55                  60

Thr Val Ile Glu Arg Pro Thr Ile Gly Ser Leu Asn Ile Thr Gly Ala
65                  70                  75                  80

Lys Met Leu Gln Asn Asp Ala Ile Lys Lys Asn Leu Glu Ser Phe Gly
                85                  90                  95

Leu Ala Gln Ser Gln Tyr Phe Asn Gln Ala Thr Leu Asn Gln Ala Val
            100                 105                 110

Ala Gly Leu Lys Glu Glu Tyr Leu Gly Arg Gly Lys Leu Asn Ile Gln
        115                 120                 125

Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn Arg Val Asp Ile Asp
    130                 135                 140

Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile Thr Asp Ile Glu Phe
145                 150                 155                 160

Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu Met Arg Gln Met Ser
                165                 170                 175

Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr Arg Ser Asp Arg Phe
            180                 185                 190

Asp Arg Gln Lys Phe Ala Gln Asp Met Glu Lys Val Thr Asp Phe Tyr
        195                 200                 205

Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu Asp Thr Asp Ile Gln
    210                 215                 220
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```
Thr Asn Glu Asp Lys Thr Arg Gln Thr Ile Lys Ile Thr Val His Glu
225                 230                 235                 240

Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile Glu Gly Asp Thr Asn
                245                 250                 255

Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu Thr Met Lys Pro Gly
            260                 265                 270

Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val Leu Gly Glu Ile Gln
        275                 280                 285

Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser Glu Ile Ser Val Gln
    290                 295                 300

Pro Leu Pro Asn Ala Gly Thr Lys Thr Val Asp Phe Val Leu His Ile
305                 310                 315                 320

Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile His Ile Thr Gly Asn
                325                 330                 335

Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu Leu Arg Gln Met Glu
            340                 345                 350

Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg Ser Lys Glu Arg Val
        355                 360                 365

Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe Asp Ala Val Pro Leu
    370                 375                 380

Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met Ser Leu Thr Glu Arg
385                 390                 395                 400

Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp Val Gln Asp Thr Gly
                405                 410                 415

Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn Leu Phe Gly Thr Gly
            420                 425                 430

Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys Thr Thr Leu Asn Gly
        435                 440                 445

Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala Asp Gly Val Ser Leu
    450                 455                 460

Gly Tyr Asp Ile Tyr Gly Lys Ala Phe Asp Pro Arg Lys Ala Ser Thr
465                 470                 475                 480

Ser Val Lys Gln Tyr Lys Thr Thr Thr Ala Gly Gly Gly Val Arg Met
                485                 490                 495

Gly Ile Pro Val Thr Glu Tyr Asp Arg Val Asn Phe Gly Leu Ala Ala
            500                 505                 510

Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala Pro Lys Arg Tyr Ala
        515                 520                 525

Asp Phe Ile Arg Lys Tyr Gly Lys Thr Asp Gly Ala Asp Gly Ser Phe
    530                 535                 540
```

```
Lys Gly Leu Leu Tyr Lys Gly Thr Val Gly Trp Gly Arg Asn Lys Thr
545                 550                 555                 560

Asp Ser Ala Ser Trp Pro Thr Arg Gly Tyr Leu Thr Gly Val Asn Ala
                565                 570                 575

Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr Tyr Ser Ala Thr His
            580                 585                 590

Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr Phe Thr Leu Met Leu
        595                 600                 605

Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly Arg Thr Lys Glu Ile
    610                 615                 620

Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu Gly Ser Val Arg Gly
625                 630                 635                 640

Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr Asp Glu Tyr Gly Glu
                645                 650                 655

Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn Val Ser Ala Glu Leu
            660                 665                 670

Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg Thr Val Arg Leu Ser
        675                 680                 685

Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly Arg Thr Tyr Thr Ala
    690                 695                 700

Ala Glu Asn Gly Asn Asn Lys Ser Val Tyr Ser Glu Asn Ala His Lys
705                 710                 715                 720

Ser Thr Phe Thr Asn Glu Leu Arg Tyr Ser Ala Gly Gly Ala Val Thr
                725                 730                 735

Trp Leu Ser Pro Leu Gly Pro Met Lys Phe Ser Tyr Ala Tyr Pro Leu
            740                 745                 750

Lys Lys Lys Pro Glu Asp Glu Ile Gln Arg Phe Gln Phe Gln Leu Gly
        755                 760                 765

Thr Thr Phe
    770

<210>  10
<211>  2394
<212>  DNA
<213>  Neisseria meningitidis

<400>  10
atgaaactga aacagattgc ttccgcactg atggtcttgg gcatatcgcc tttggcactt    60
gccgacttca ccatccaaga catccgcgtc gaaggcttgc agcgtaccga gccgagtacc   120
gtattcaact acctgcccgt caaagtcggc gacacctaca acgacacaca cggcagtgcc   180
atcatcaaaa gcctgtacgc caccggtttc tttgacgacg tacgcgtcga aactgcggac   240
gggcagctcc tgctgaccgt tatcgaacgc cccaccatcg gctcgctcaa catcaccggc   300
gcaaaaatgc tgcaaaacga cgccattaag aaaaacctcg aatcgttcgg gctggcgcag   360
tcgcaatact ttaatcaggc gacactcaat caggcagtcg ccggcctgaa agaagaatac   420
ctcgggcgcg gcaaactcaa tatccaaatc acgcccaaag taaccaaact cgcccgcaac   480
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cgcgtcgaca tcgacatcac gattgacgag ggcaaatccg ccaaaatcac cgacatcgaa   540
tttgaaggca accaagtcta ttccgaccgc aaactgatgc ggcagatgtc gctgaccgaa   600
ggcggcattt ggacatggct gacacgaagc aaccaattca acgagcagaa atttgcccaa   660
gacatggaaa aagtaaccga cttctaccag aacaacggct acttcgattt ccgcatcctc   720
gataccgaca tccaaaccaa cgaagacaaa accaagcaga ccatcaaaat caccgtccac   780
gaaggcggac gtttccgttg gggcaaagtc tccatcgaag gcgacaccaa cgaagtcccc   840
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gaaatcagcg tacagccgct gccaaacgcc gaaaccaaaa ccgtcgattt cgtcctgcac  1020
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cgcgacgaag tcgtgcgccg cgaattgcgc caaatggaat ccgcgcctta cgacacctcc  1140
aagctgcaac gctccaaaga gcgcgtcgag cttttgggct acttcgacaa cgtacagttt  1200
gatgccgtcc cgcttgccgg cacacccgac aaagtcgatt tgaacatgag cctgaccgaa  1260
cgttccaccg gctcgctcga cttgagcgcg ggctgggtac aggataccgg cctggtcatg  1320
tccgcaggcg tttcccaaga caacctgttc ggtacgggca agtcggccgc cctgcgcgcc  1380
tcacgaagca aaaccacgct caacggctcg ctgtcgttta ccgacccgta cttcacggca  1440
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gttacctggc tctcgccttt aggcccgatg aaattcagct acgcctaccc gctgaagaaa  2340
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<212>  PRT
<213>  Neisseria meningitidis

<400>  11
Met Lys Leu Lys Gln Ile Ala Ser Ala Leu Met Val Leu Gly Ile Ser
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Pro Leu Ala Leu Ala Asp Phe Thr Ile Gln Asp Ile Arg Val Glu Gly
            20                  25                  30

Leu Gln Arg Thr Glu Pro Ser Thr Val Phe Asn Tyr Leu Pro Val Lys
        35                  40                  45

Val Gly Asp Thr Tyr Asn Asp Thr His Gly Ser Ala Ile Ile Lys Ser
    50                  55                  60

Leu Tyr Ala Thr Gly Phe Phe Asp Asp Val Arg Val Glu Thr Ala Asp
65                  70                  75                  80

Gly Gln Leu Leu Leu Thr Val Ile Glu Arg Pro Thr Ile Gly Ser Leu
                85                  90                  95

Asn Ile Thr Gly Ala Lys Met Leu Gln Asn Asp Ala Ile Lys Lys Asn
            100                 105                 110
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Leu Glu Ser Phe Gly Leu Ala Gln Ser Gln Tyr Phe Asn Gln Ala Thr
        115                 120                 125

Leu Asn Gln Ala Val Ala Gly Leu Lys Glu Glu Tyr Leu Gly Arg Gly
    130                 135                 140

Lys Leu Asn Ile Gln Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn
145                 150                 155                 160

Arg Val Asp Ile Asp Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile
                165                 170                 175

Thr Asp Ile Glu Phe Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu
            180                 185                 190

Met Arg Gln Met Ser Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr
        195                 200                 205

Arg Ser Asn Gln Phe Asn Glu Gln Lys Phe Ala Gln Asp Met Glu Lys
    210                 215                 220

Val Thr Asp Phe Tyr Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu
225                 230                 235                 240

Asp Thr Asp Ile Gln Thr Asn Glu Asp Lys Thr Lys Gln Thr Ile Lys
                245                 250                 255

Ile Thr Val His Glu Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile
            260                 265                 270

Glu Gly Asp Thr Asn Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu
        275                 280                 285

Thr Met Lys Pro Gly Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val
    290                 295                 300

Leu Gly Glu Ile Gln Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser
305                 310                 315                 320

Glu Ile Ser Val Gln Pro Leu Pro Asn Ala Glu Thr Lys Thr Val Asp
                325                 330                 335

Phe Val Leu His Ile Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile
            340                 345                 350

His Ile Thr Gly Asn Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu
        355                 360                 365

Leu Arg Gln Met Glu Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg
    370                 375                 380

Ser Lys Glu Arg Val Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe
385                 390                 395                 400

Asp Ala Val Pro Leu Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met
                405                 410                 415

Ser Leu Thr Glu Arg Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp
            420                 425                 430
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Val Gln Asp Thr Gly Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn
        435                 440                 445

Leu Phe Gly Thr Gly Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys
    450                 455                 460

Thr Thr Leu Asn Gly Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala
465                 470                 475                 480

Asp Gly Val Ser Leu Gly Tyr Asp Val Tyr Gly Lys Ala Phe Asp Pro
                485                 490                 495

Arg Lys Ala Ser Thr Ser Ile Lys Gln Tyr Lys Thr Thr Thr Ala Gly
            500                 505                 510

Ala Gly Ile Arg Met Ser Val Pro Val Thr Glu Tyr Asp Arg Val Asn
        515                 520                 525

Phe Gly Leu Val Ala Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala
    530                 535                 540

Pro Lys His Tyr Ala Asp Phe Ile Lys Lys Tyr Gly Lys Thr Asp Gly
545                 550                 555                 560

Thr Asp Gly Ser Phe Lys Gly Trp Leu Tyr Lys Gly Thr Val Gly Trp
                565                 570                 575

Gly Arg Asn Lys Thr Asp Ser Ala Leu Trp Pro Thr Arg Gly Tyr Leu
            580                 585                 590

Thr Gly Val Asn Ala Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr
        595                 600                 605

Tyr Ser Ala Thr His Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr
    610                 615                 620

Phe Thr Leu Met Leu Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly
625                 630                 635                 640

Arg Thr Lys Glu Ile Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu
                645                 650                 655

Gly Ser Val Arg Gly Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr
            660                 665                 670

Asp Glu Tyr Gly Glu Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn
        675                 680                 685

Val Ser Ala Glu Leu Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg
    690                 695                 700

Thr Val Arg Leu Ser Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly
705                 710                 715                 720

Lys Thr Tyr Asp Asp Asn Ser Ser Ser Ala Thr Gly Gly Arg Val Gln
                725                 730                 735

Asn Ile Tyr Gly Ala Gly Asn Thr His Lys Ser Thr Phe Thr Asn Glu
            740                 745                 750
```

```
Leu Arg Tyr Ser Ala Gly Gly Ala Val Thr Trp Leu Ser Pro Leu Gly
        755                 760                 765

Pro Met Lys Phe Ser Tyr Ala Tyr Pro Leu Lys Lys Lys Pro Glu Asp
    770                 775                 780

Glu Ile Gln Arg Phe Gln Phe Gln Leu Gly Thr Thr Phe
785                 790                 795

<210>   12
<211>   21
<212>   PRT
<213>   Neisseria meningitidis

<400>   12
Met Lys Leu Lys Gln Ile Ala Ser Ala Leu Met Val Leu Gly Ile Ser
1               5                   10                  15

Pro Leu Ala Leu Ala
            20

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<212>   PRT
<213>   Neisseria meningitidis

<400>   13
Asp Phe Thr Ile Gln Asp Ile Arg Val Glu Gly Leu Gln Arg Thr Glu
1               5                   10                  15

Pro Ser Thr Val Phe Asn Tyr Leu Pro Val Lys Val Gly Asp Thr Tyr
            20                  25                  30

Asn Asp Thr His Gly Ser Ala Ile Ile Lys Ser Leu Tyr Ala Thr Gly
        35                  40                  45

Phe Phe Asp Asp Val Arg Val Glu Thr Ala Asp Gly Gln Leu Leu Leu
    50                  55                  60

Thr Val Ile Glu Arg Pro Thr Ile Gly Ser Leu Asn Ile Thr Gly Ala
65                  70                  75                  80

Lys Met Leu Gln Asn Asp Ala Ile Lys Lys Asn Leu Glu Ser Phe Gly
                85                  90                  95

Leu Ala Gln Ser Gln Tyr Phe Asn Gln Ala Thr Leu Asn Gln Ala Val
            100                 105                 110

Ala Gly Leu Lys Glu Glu Tyr Leu Gly Arg Gly Lys Leu Asn Ile Gln
        115                 120                 125

Ile Thr Pro Lys Val Thr Lys Leu Ala Arg Asn Arg Val Asp Ile Asp
    130                 135                 140

Ile Thr Ile Asp Glu Gly Lys Ser Ala Lys Ile Thr Asp Ile Glu Phe
145                 150                 155                 160

Glu Gly Asn Gln Val Tyr Ser Asp Arg Lys Leu Met Arg Gln Met Ser
                165                 170                 175
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Leu Thr Glu Gly Gly Ile Trp Thr Trp Leu Thr Arg Ser Asn Gln Phe
            180                 185                 190

Asn Glu Gln Lys Phe Ala Gln Asp Met Glu Lys Val Thr Asp Phe Tyr
        195                 200                 205

Gln Asn Asn Gly Tyr Phe Asp Phe Arg Ile Leu Asp Thr Asp Ile Gln
    210                 215                 220

Thr Asn Glu Asp Lys Thr Lys Gln Thr Ile Lys Ile Thr Val His Glu
225                 230                 235                 240

Gly Gly Arg Phe Arg Trp Gly Lys Val Ser Ile Glu Gly Asp Thr Asn
                245                 250                 255

Glu Val Pro Lys Ala Glu Leu Glu Lys Leu Leu Thr Met Lys Pro Gly
            260                 265                 270

Lys Trp Tyr Glu Arg Gln Gln Met Thr Ala Val Leu Gly Glu Ile Gln
        275                 280                 285

Asn Arg Met Gly Ser Ala Gly Tyr Ala Tyr Ser Glu Ile Ser Val Gln
    290                 295                 300

Pro Leu Pro Asn Ala Glu Thr Lys Thr Val Asp Phe Val Leu His Ile
305                 310                 315                 320

Glu Pro Gly Arg Lys Ile Tyr Val Asn Glu Ile His Ile Thr Gly Asn
                325                 330                 335

Asn Lys Thr Arg Asp Glu Val Val Arg Arg Glu Leu Arg Gln Met Glu
            340                 345                 350

Ser Ala Pro Tyr Asp Thr Ser Lys Leu Gln Arg Ser Lys Glu Arg Val
        355                 360                 365

Glu Leu Leu Gly Tyr Phe Asp Asn Val Gln Phe Asp Ala Val Pro Leu
    370                 375                 380

Ala Gly Thr Pro Asp Lys Val Asp Leu Asn Met Ser Leu Thr Glu Arg
385                 390                 395                 400

Ser Thr Gly Ser Leu Asp Leu Ser Ala Gly Trp Val Gln Asp Thr Gly
                405                 410                 415

Leu Val Met Ser Ala Gly Val Ser Gln Asp Asn Leu Phe Gly Thr Gly
            420                 425                 430

Lys Ser Ala Ala Leu Arg Ala Ser Arg Ser Lys Thr Thr Leu Asn Gly
        435                 440                 445

Ser Leu Ser Phe Thr Asp Pro Tyr Phe Thr Ala Asp Gly Val Ser Leu
    450                 455                 460

Gly Tyr Asp Val Tyr Gly Lys Ala Phe Asp Pro Arg Lys Ala Ser Thr
465                 470                 475                 480

Ser Ile Lys Gln Tyr Lys Thr Thr Thr Ala Gly Ala Gly Ile Arg Met
                485                 490                 495
```

```
Ser Val Pro Val Thr Glu Tyr Asp Arg Val Asn Phe Gly Leu Val Ala
            500                 505                 510

Glu His Leu Thr Val Asn Thr Tyr Asn Lys Ala Pro Lys His Tyr Ala
        515                 520                 525

Asp Phe Ile Lys Lys Tyr Gly Lys Thr Asp Gly Thr Asp Gly Ser Phe
    530                 535                 540

Lys Gly Trp Leu Tyr Lys Gly Thr Val Gly Trp Gly Arg Asn Lys Thr
545                 550                 555                 560

Asp Ser Ala Leu Trp Pro Thr Arg Gly Tyr Leu Thr Gly Val Asn Ala
                565                 570                 575

Glu Ile Ala Leu Pro Gly Ser Lys Leu Gln Tyr Tyr Ser Ala Thr His
            580                 585                 590

Asn Gln Thr Trp Phe Phe Pro Leu Ser Lys Thr Phe Thr Leu Met Leu
        595                 600                 605

Gly Gly Glu Val Gly Ile Ala Gly Gly Tyr Gly Arg Thr Lys Glu Ile
    610                 615                 620

Pro Phe Phe Glu Asn Phe Tyr Gly Gly Gly Leu Gly Ser Val Arg Gly
625                 630                 635                 640

Tyr Glu Ser Gly Thr Leu Gly Pro Lys Val Tyr Asp Glu Tyr Gly Glu
                645                 650                 655

Lys Ile Ser Tyr Gly Gly Asn Lys Lys Ala Asn Val Ser Ala Glu Leu
            660                 665                 670

Leu Phe Pro Met Pro Gly Ala Lys Asp Ala Arg Thr Val Arg Leu Ser
        675                 680                 685

Leu Phe Ala Asp Ala Gly Ser Val Trp Asp Gly Lys Thr Tyr Asp Asp
    690                 695                 700

Asn Ser Ser Ser Ala Thr Gly Gly Arg Val Gln Asn Ile Tyr Gly Ala
705                 710                 715                 720

Gly Asn Thr His Lys Ser Thr Phe Thr Asn Glu Leu Arg Tyr Ser Ala
                725                 730                 735

Gly Gly Ala Val Thr Trp Leu Ser Pro Leu Gly Pro Met Lys Phe Ser
            740                 745                 750

Tyr Ala Tyr Pro Leu Lys Lys Lys Pro Glu Asp Glu Ile Gln Arg Phe
        755                 760                 765

Gln Phe Gln Leu Gly Thr Thr Phe
    770                 775

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<211>  441
<212>  PRT
<213>  Neisseria meningitidis

<400>  14
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Met Lys Lys Tyr Leu Phe Arg Ala Ala Leu Tyr Gly Ile Ala Ala Ala
1               5                   10                  15

Ile Leu Ala Ala Cys Gln Ser Lys Ser Ile Gln Thr Phe Pro Gln Pro
            20                  25                  30

Asp Thr Ser Val Ile Asn Gly Pro Asp Arg Pro Val Gly Ile Pro Asp
        35                  40                  45

Pro Ala Gly Thr Thr Val Gly Gly Gly Gly Ala Val Tyr Thr Val Val
    50                  55                  60

Pro His Leu Ser Leu Pro His Trp Ala Ala Gln Asp Phe Ala Lys Ser
65                  70                  75                  80

Leu Gln Ser Phe Arg Leu Gly Cys Ala Asn Leu Lys Asn Arg Gln Gly
                85                  90                  95

Trp Gln Asp Val Cys Ala Gln Ala Phe Gln Thr Pro Val His Ser Phe
            100                 105                 110

Gln Ala Lys Gln Phe Phe Glu Arg Tyr Phe Thr Pro Trp Gln Val Ala
        115                 120                 125

Gly Asn Gly Ser Leu Ala Gly Thr Val Thr Gly Tyr Tyr Glu Pro Val
    130                 135                 140

Leu Lys Gly Asp Asp Arg Arg Thr Ala Gln Ala Arg Phe Pro Ile Tyr
145                 150                 155                 160

Gly Ile Pro Asp Asp Phe Ile Ser Val Pro Leu Pro Ala Gly Leu Arg
                165                 170                 175

Ser Gly Lys Ala Leu Val Arg Ile Arg Gln Thr Gly Lys Asn Ser Gly
            180                 185                 190

Thr Ile Asp Asn Thr Gly Gly Thr His Thr Ala Asp Leu Ser Arg Phe
        195                 200                 205

Pro Ile Thr Ala Arg Thr Thr Ala Ile Lys Gly Arg Phe Glu Gly Ser
    210                 215                 220

Arg Phe Leu Pro Tyr His Thr Arg Asn Gln Ile Asn Gly Gly Ala Leu
225                 230                 235                 240

Asp Gly Lys Ala Pro Ile Leu Gly Tyr Ala Glu Asp Pro Val Glu Leu
                245                 250                 255

Phe Phe Met His Ile Gln Gly Ser Gly Arg Leu Lys Thr Pro Ser Gly
            260                 265                 270

Lys Tyr Ile Arg Ile Gly Tyr Ala Asp Lys Asn Glu His Pro Tyr Val
        275                 280                 285

Ser Ile Gly Arg Tyr Met Ala Asp Lys Gly Tyr Leu Lys Leu Gly Gln
    290                 295                 300

Thr Ser Met Gln Gly Ile Lys Ala Tyr Met Arg Gln Asn Pro Gln Arg
305                 310                 315                 320
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Leu Ala Glu Val Leu Gly Gln Asn Pro Ser Tyr Ile Phe Phe Arg Glu
                325                 330                 335

Leu Ala Gly Ser Ser Asn Asp Gly Pro Val Gly Ala Leu Gly Thr Pro
            340                 345                 350

Leu Met Gly Glu Tyr Ala Gly Ala Val Asp Arg His Tyr Ile Thr Leu
        355                 360                 365

Gly Ala Pro Leu Phe Val Ala Thr Ala His Pro Val Thr Arg Lys Ala
    370                 375                 380

Leu Asn Arg Leu Ile Met Ala Gln Asp Thr Gly Ser Ala Ile Lys Gly
385                 390                 395                 400

Ala Val Arg Val Asp Tyr Phe Trp Gly Tyr Gly Asp Glu Ala Gly Glu
                405                 410                 415

Leu Ala Gly Lys Gln Lys Thr Thr Gly Tyr Val Trp Gln Leu Leu Pro
            420                 425                 430

Asn Gly Met Lys Pro Glu Tyr Arg Pro
        435                 440
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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition comprising (a) outer membrane vesicles from *N.meningitidis* serogroup B; and (b) an immunogenic component comprising (i) an amino acid sequence selected from the group consisting of
MFKRSVIAMACIFPLSACGGGGGGSPDVKSADTPSKPAAPVVAENAGEGVLPKEKKDEEAAGGAPQADTQDA
TAGEGSQDMAAVSAENTGNGGAATTDNPKNEDAGAQNDMPQNAAESANQTGNNQPAGSSDSAPASNPAPA
NGGSDFGRTNVGNSVVIDGPSQNITLTHCKGDSCNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGL
VADRVKKDGTNKYIIFYTDKPPTRSARSRRSLPAEIPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTY
GAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGSKSVDGIIDSGDDL
HMGTQKFKAAIDGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKDRD,
MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGSQDM
AAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGTDSSTPNHTPDPNMLAGNMENQATDAGESSQPAN
QPDMANAADGMQGDDPSAGGQNAGNTAAQGANQAGNNQAAGSSDPIPASNPAPANGGSNFGRVDLANGVL
IDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDKFVGLVADSVQMKGINQYIIF
YKPKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSY
ALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKA
AIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD,
and
MFKRSVIAMACIVALSACGGGGGGSPDVKSADTLSKPAAPVVTEDVGEEVLPKEKKDEEAVSGAPQADTQDA
TAGKGGQDMAAVSAENTGNGGAATTDNPENKDEGPQNDMPQNAADTDSSTPNHTPAPNMPTRDMGNQAPD
AGESAQPANQPDMANAADGMQGDDPSAGENAGNTADQAANQAENNQVGGSQNPASSTNPNATNGGSDFGR
INVANGIKLDSGSENVTLTHCKDKVCDRDFLDEEAPPKSEFEKLSDEEKINKYKKDEQRENFVGLVADRVEKN
GTNKYVIIYKDKSASSSSARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYG
AEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDFGSKSVDGIIDSGDDLH
MGTQKFKAVIDGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD, (ii) a fragment of 10 or more contiguous amino acids of (i), and/or (iii) an amino acid sequence having 70% or more sequence identity to (i).

2. The composition of claim 1, wherein component (b) includes a protein from a different *N.meningitidis* serogroup B strain from that from which component (a) is derived.

3. The composition of claim 1 or 2, wherein the outer membrane vesicles (OMVs) are a deoxycholate extract from NmB.

4. The composition of any one of claims 1 to 3, wherein one or more of its components is adsorbed on Al(OH)$_3$.

5. The composition of claim 4, wherein component (a) is adsorbed on $Al(OH)_3$.

6. The composition of any one of claims 1 to 5, further comprising one or more of the following components:

- a protective antigen against *Neisseria meningitidis* serogroup A;
- a protective antigen against *Neisseria meningitidis* serogroup C;
- a protective antigen against *Neisseria meningitidis* serogroup Y;
- a protective antigen against *Neisseria meningitidis* serogroup W;
- a protective antigen against *Haemophilus influenzae*;
- a protective antigen against *pneumococcus*;
- a protective antigen against diphtheria;
- a protective antigen against tetanus;
- a protective antigen against whooping cough;
- a protective antigen against *Helicobacter pylori*;
- a protective antigen against polio; and/or
- a protective antigen against hepatitis B virus.

7. The composition of any one of claims 1 to 6, wherein the composition is a vaccine.

8. The composition of any one of claims 1 to 7, for use as a medicament.

9. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to any one of claims 1 to 8.

10. Use of the composition according to any one of claims 1 to 8 in medicine.

11. Use of the composition according to any one of claims 1 to 8 in the manufacture of a medicament for the prophylactic or therapeutic treatment of a patient.

12. The composition of any one of claims 1 to 8 substantially as described herein with reference to any one or more of the Examples.

13. The method of claim 9 substantially as described herein with reference to any one or more of the Examples.

14. The use of claim 10 or 11 substantially as described herein with reference to any one or more of the Examples.

Dated this SEVENTH day of FEBRUARY 2006

Chiron S.p.A.
Patent Attorneys for the Applicant:

F B RICE & CO