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REVIEW

Capsular Antigens of Bacteria. Capsular Antigens as the Basis of Vaccines against Pathogenic Bacteria

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Abstract—The role of bacterial capsular antigens represented in capsular polysaccharides and exoglycans in pathogenicity and virulence of bacteria is discussed in this review. Using capsular antigens for vaccines against severe diseases caused by capsular microorganisms is considered in detail. The use of conjugates of capsular polysaccharides and their fragments with proteins and peptides for vaccine as well as using liposomes as adjuvants for the capsular antigens are described. Data concerning structural elucidation of bacterial capsular antigens are given in the first part of this review.

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CAPSULAR ANTIGENS AS FACTORS OF BACTERIAL PATHOGENICITY AND VIRULENCE

Pathogenicity. That the interrelation of structural features of capsular antigens involving capsular polysaccharides and exoglycans and bacterial pathogenicity causes all the symptoms of disease is without doubt [1]. *Neisseria meningitidis* groups B and C and *Escherichia coli* K1 produce the same homopolymer of α-D-(2→8)-linked sialic acid as their single common surface component, and both microorganisms are the main causative factors of infant meningitis. Pathogenicity of bacteria arises from the ability of the capsular antigens to inhibit activation of complement by the alternative pathway. One structural feature of the capsular antigens is known to bear terminal sialic acid residues. The ability of the terminal sialic acid residues to inhibit activation of the alternate complement pathway proved to be peculiar for surface membrane erythrocytes. Streptococci group B type III are known to be powerful inhibitors of the alternate pathway of complement activation; however, these microbes are converted into activators of the alternate pathway after preliminary reduction of carboxyl groups or using growth on medium with neuraminidase releasing the terminal residues of sialic acid. In this case, activation of the host immune system is observed in response to microorganism pathogenicity.

Virulence. Humans are constantly in contact with a substantial diversity of microorganisms in the environment, but only a few of them are virulent. The bacterial virulence depends on the ability to penetrate into the human organism and to cause a response of the host immune system. The capsular polysaccharides proved to be important agents in bacterial virulence due to their localization on the bacterial surface and direct interaction with the host immune system [1]. A primary step of virulence of the majority of bacterial infections is attachment of bacteria to mucous membrane. The capsular polysaccharides are not involved in this mechanism; however, they participate very actively in the second step of bacterial virulence promoting penetration of these bacteria in body tissues. Numerous data demonstrate that the capsular antigens represent very important factors of virulence in diseases caused by the following microorganisms: *N. meningitidis*, *Haemophilus influenzae*, *Streptococcus* group B, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* (see, e.g. [2]). The significance of the capsular polysaccharides in pneumococcus infection was shown when enzymic depolymerization of the capsular polysaccharide on the surface of pneumococcus *S. pneumoniae* type 3 was found to decrease considerably the virulence of the microbe. A property of the capsular polysaccharide causing intensity of virulence of the microorganisms is its ability to inhibit the host immune system. The main mechanism of this interaction is expressed by a function of the

capsular polysaccharide as inhibitor of the rapid alternate pathway of complement activation to oblige the host immune system to use the slower classic pathway of compliment activation [1].

Virulence as a measure of pathogenicity and ability of a microorganism to transfer from one host to another, causing symptoms of the disease, is as a rule in proportion to the amount of capsular polysaccharide, although exceptions to the rule occur. Thus, for example, pneumococcus *S. pneumoniae* type 3 possessing a large capsule is exclusively virulent, while *S. pneumoniae* type 37 possessing approximately the same capsule fails to possess virulence; however, pneumococci type 12 with small capsule show very high virulence [1].

CAPSULAR ANTIGENS AS THE BASIS OF VACCINATION

Infectious diseases caused by capsular bacteria are widespread and severe bacterial infections throughout the World. The capsular antigens produced by these microorganisms often possess high immunogenicity and constant attempts are made to use them as components of vaccines against diseases caused by capsular microbes (see, for example, reviews [1, 3-6]).

Staphylococcus aureus. Vaccines on the basis of the capsular polysaccharide from *S. aureus* type 5 (CP5) cause production of a high level of antibodies, especially immunoglobulin IgG. More satisfactory results are observed on immunization with whole microbial cells expressing CP5 [7].

The purified capsular polysaccharides of serotypes 5 and 8 were selected as the basic antigens for prevention of staphylococcus infection, although other polysaccharides may play a substantial role in the creation of effective vaccine [8, 9].

Streptococcus pneumoniae. Pneumonia remains one of the most important causative factors of mortality connected with infections of the respiratory tract of adults and, additionally, with otitis of children (bacterial infections of the middle ear). The high mortality caused by pneumonia motivated the search for protective methods for monitoring this disease and investigation of the pneumococcal capsular polysaccharides as the first purified polysaccharides used as vaccines for humans. The classic research of the American immunochemist M. Heidelberger [10-12] led to the conclusion that vaccines on the basis of polysaccharides of the pneumococcal capsular antigens are effective in relation to pneumonia caused by the pathogenic microorganisms *S. pneumoniae* (*Diplococcus pneumoniae*).

In 1930, the important discovery [13] was made that subcutaneous injection of some pneumococcal polysaccharides induced production of human antibodies against the causative agent of the disease. Attempts to use mixtures of capsular polysaccharides from various pneumococcal types as so-called polyvalent vaccines were successful. Thus, for example, six-valent polysaccharide vaccine in a single injection induces the necessary level of antibodies, which lasts for eight years [14].

This success led very quickly to a commercial license for the six-valent vaccine on the basis of pneumococcal polysaccharides. In the period of World War II, M. Heidelberger immunized a large group of American soldiers with polysaccharide vaccines against pneumonia to prevent this disease, which was severe at that time. However, at the same time, interest in prophylaxis of pneumonia was weakened due to successful curing with sulfamide preparations and later in connection with unprecedented success of therapy with antibiotics. Even serotyping of excretions during disease was stopped in the majority of medical centers. However, subsequent epidemiological investigations [15] of pneumococcal pneumonia showed that the disease occurs with the same frequency and with the same level of mortality as before using antibiotics in spite of success of therapy with antibiotics. This phenomenon together with the danger of appearance of new pneumococcal strains resistant to antibiotics led to a changed strategy for defense from the disease, namely, the interrupted investigations for using and license of vaccines on the basis of pneumococcal polysaccharides were continued.

Seven-valent vaccine containing capsular polysaccharides of serotypes 1, 4, 5, 7F, 9V, 19F, and 23F was introduced for vaccination. To cover as many as possible pneumococcal serotypes with a vaccination, 11-valent vaccine containing all the capsular antigens of the seven-valent vaccines conjugated with a tetanic protein together with polysaccharides of serotypes 3, 6B, 14, and 18C conjugated with diphtheria toxin [16] was proposed for vaccination of children. The vaccine was used for immunization of infants up to one-year-old in the national programs of Finland and Israel. The vaccine did not cause collateral effects and was satisfactorily immunogenic. Thus, the natural conclusion was made that the vaccine may be widely used, especially in the cases when the regular seven-valent vaccine was insufficiently effective [16].

It is interesting to note that substantial differences are observed in immunogenicity of each capsular polysaccharide included in seven-valent vaccine [17, 18]. The production of antibodies to the capsular pneumococcal polysaccharides and their levels are in direct dependence on serotypes [18].

Due to diversity of pneumococcal types, 23-valent vaccine created based on the data about distribution and spreading of serotypes and concerning cross-reactions between various serotypes of pneumococcus has been widely used since 1983 [3, 19-22].

In the technology of production of vaccines based on conjugates of pneumococcal capsular polysaccharides, the following methods of fragmentation are used: partial

depolymerization with ultrasound or an electron beam. This is followed by conjugation with a tetanic toxoid. These methods are notably effective and simple and provide various conjugates of polysaccharides with proteins containing fragments of pneumococcal capsular polysaccharides of different types, which also differ in size and substitution. These are characterized by a higher immunogenicity [23].

Vaccines based on the capsular polysaccharides of pneumococcus produce type-specific immunoglobulins IgG, IgM, and IgA, but only IgG causes effective defense against homological serotypes of pneumococcus; type-specific IgM and IgA impart a certain contribution in this defense by modulating inflammatory response to pneumococci [24-26].

It must be noted that immunoglobulin IgG stimulated by a vaccine in pneumonia patients in comparison with healthy adults possesses much lower ability to opsonize the infections pneumococcal serotype for phagocytosis *in vitro* with normal polymorphonuclear leucocytes or to protect mice from experimental infection [27].

Very interesting data were obtained by immunization of nursing mothers with 23-valent polysaccharide vaccine against pneumococci [28]. The concentration of specific immunoglobulin IgA (but not IgG) in milk increase more than twofold, and this was observed to destroy pneumococci and to confirm that the specific immunoglobulin IgA in woman's milk supports functional bactericidal activity and strengthen defense of infants against diseases caused by pneumococci [28].

Neisseria meningitidis. Meningitis often occurs in children, but it is also observed in adults. Groups A, B, and C of eight serogroups of *N. meningitidis* appear to be the causative factors of more than 90% of meningitis.

The main problem with three-valent vaccine is low immunogenicity of polysaccharide of *N. meningitidis* group B for humans [29]. This polysaccharide contains α-D(2$\rightarrow$8)-linked homopolymer of sialic acid, which is quickly depolymerized in human tissues by neuraminidase. In addition, this structure is recognized by the human immune system as "self" and, therefore, antibodies were not formed against this structure.

Glycoconjugate vaccines to group A and C are safe for children and give rise to specific anti-A and anti-C antibodies with satisfactory titers on the primary immunization [1, 3].

O-Acetyl groups as additional specific epitopes play a substantial role in forming of functional immune response to the capsular polysaccharide of *N. meningitidis* serogroup A [30]. O-Acetyl groups in immunogenic epitopes of capsular polysaccharide appear to be very important for protective action of vaccine.

Haemophilus influenzae. In spite of the existence of six types of *H. influenzae* [1, 31], only type *b* causes the most severe meningitis exclusively in children, and even after recovery from this disease very serious neurological complications may occur. Using polysaccharide of this *H. influenzae* type together with complement, long-living antibodies can be induced in human adults, and the capsular antigen of this type is used as the main constituent of the vaccine against meningitis [1]. However, elaboration of this vaccine was suspended when the polysaccharide was found to induce only short-living antibodies of older children and failed to induce antibodies or produce them in negligible amounts in infants [32].

In the early 1990s, conjugated vaccines were introduced into medical practice [33], and this resulted in a substantial decrease in meningitis in Finland, Great Britain, and the USA with very high efficacy for infants as follows: 99, 97, and 94% for infants up to 1-year-, 1-2-year-, and 2-3-year-old, respectively [34].

Clear response to capsular polysaccharide with forming of specific antibodies is observed in infants on immunization with vaccines based on capsular polysaccharides conjugated with a protein carrier; the same effect was found in immunization of elderly people with conjugate of polysaccharide with diphtheria toxin detoxified by previous treatment [1, 3, 35, 36].

***Streptococcus* group B.** These microorganisms represent the main causative factor of bacterial meningitis in newborns [1]. Only the type III of five known types of streptococcus group B is the most important in etiology of this disease in children. The complete capsular antigen of this type plays a substantial role in immunity, but an incomplete antigen lacking the terminal residue of sialic acid is usually obtained. Using such antigen in immunization demonstrated that antibodies obtained can play a protective role against streptococci group B, type III for older children.

The use of such vaccine for newborns is connected with immunization of the expectant mother using native polysaccharide. Polysaccharide of type III is immunogenic for adults and as a result the child is immunized by transfer of antibodies through the placenta. Children born from immunized mothers were shown to be less exposed to infection than others [37].

CONJUGATES OF CAPSULAR ANTIGENS AND THEIR SEGMENTS AS VACCINES

Application of vaccines based on capsular polysaccharides and exoglycans leads to only partial success because they are poor immunogens for infants, the elderly, and immunodeficient patients who are less exposed to diseases caused by encapsulated bacteria. This is connected with T-independence of the capsular antigens [38, 39]. A solution for this problem of augmentation of the capsular polysaccharide immunogenicity is accompanied by their conversion into thymus-dependent antigens by conjugation of the purified polysaccharide antigens with protein carrier.

Conjugates of polysaccharides with proteins. Blood erythrocytes, horse serum globulins, BSA, etc. are used as protein carriers.

In the early 1930s, the outstanding immunochemists W. F. Goebel (Germany) and O. T. Avery (USA) carrying out joint research [40, 41] achieved conjugation of capsular polysaccharide of pneumococci type 3 with horse serum albumin. As a result of immunization of rabbits with this conjugate, generation of antibodies was observed while immune response to the purified polysaccharide was absent. Later, many procedures of conjugation of polysaccharides with proteins were worked out on the basis of activation of the polysaccharide functional groups. For example, the following procedures used widely in practice [42] for conjugation of the capsular antigens with proteins have been described:

– polysaccharide (18.2 mg) and hemolysin (12.7 mg) are treated with sodium cyanoborohydride ($NaBH_3CN$, 9.4 mg) in 0.2 M potassium phosphate, pH 7 (3.6 ml), for 13 days at room temperature. The resulting mixture is chromatographed on a column with Sepharose CL-4B for removal of reagent excess and for preliminary purification of the conjugate;

– polysaccharide (22 mg) and hemolysin (22 mg) are mixed with 21 mM solution of glutaraldehyde in 0.1 M aqueous sodium phosphate (pH 7). The mixture obtained is kept at room temperature for 24 h under constant mixing followed by dialysis against phosphate buffer, centrifugation at 4000*g* for 5 min, and lyophilization. The resulting conjugate is analyzed by HPLC;

– polysaccharide–protein conjugates are purified by HPLC on an Ultropac TSK G 2000 SW column using elution with 10 mM sodium phosphate (pH 7) containing 10 mM NaCl. A control is carried out by determining absorption at 206 nm. Fractions containing conjugate are tested using immunoenzyme analysis (ELISA) with antiserum against whole bacterial cells. Thus, for example, vaccine possessing high immunogenicity is obtained by conjugation of the capsular polysaccharide of streptococci (type II, group B) causing ~15% of streptococcal infections with tetanic toxoid, while the vaccine based on the parent polysaccharide shows much lower activity. Immune response to the conjugated vaccine is dose-dependent and correlates with phagocytosis *in vitro*. These data demonstrate that the conjugated vaccine can be applied against streptococcal infections [43].

Procedures for conjugation by some methods are too severe for highly susceptible polysaccharides used in vaccines. A very refined method of conjugation was proposed by American researcher Prof. H. J. Jennings [1]. A controlled periodate oxidation of polysaccharide antigens gives rise to free aldehyde groups which can be used to conjugate polysaccharide with protein by specifically reductive amination with sodium cyanoborohydride. The resulting conjugates were found to be adequate immunogens for rabbits: the high titer of antibodies with specific bacterial activity against homologous microorganisms indicates the possibility of application of these immunogens as artificial synthetic vaccines on the basis of the capsular antigens.

An intensification of immune response to capsular polysaccharides after their conjugation with proteins is caused by T-helper cells (Th-cells) specific to the protein [44, 45]. Conjugation of the bacterial capsular polysaccharides with proteins leads to increasing immunogenicity of the capsular antigens and generation of antigens with new immunological features. The conjugates with proteins represent thymus-dependent antigens, while the capsular polysaccharides are thymus-independent capsular antigens. The conjugates are immunogenic for children, and they induce immunological memory and strengthen immune response with forming antibodies, especially immunoglobulin G (IgG) by B-cells [46]. These properties of conjugates are caused by T-cells due to binding protein of the conjugate with specific epitopes of T-helper cells resulting in generation of a new antigen. However, polysaccharide conjugates with peptides possess some practical advantages in comparison with polysaccharide–protein conjugates and proved to be more attractive as vaccine components [47]. Peptides for conjugation with K-antigens are selected using their potential ability to activate Th-cells, in particular, peptides of *Mycobacterium bovis* protein (hsp65, heat shock protein with molecular mass of 65 kD), etc. [44]. The conjugates of polysaccharides with peptides are of great interest for basic research as well as for applied investigations for production of effective vaccine: as the number of vaccines grows, so does the necessity for effective and safe universal protein and peptide carriers.

Experiments on animals demonstrate that antibodies against the pneumococcal capsular polysaccharides stimulate bacterial phagocytosis, as well as anti-pneumolysin antibodies possess anti-inflammatory action and prevent invasion of pneumococci. Simultaneously, both types of antibodies can play a protective role in early steps of pneumococcal infection and together they represent a vaccine for ensured effective defense in relation to *S. pneumoniae* [48-51]. In addition, capsular polysaccharides induce production of cytokines as follows: IL-4, IL-6, IL-10, and γ-interferon (IFNγ).

On the other hand, satisfactory results in production and application of vaccines were observed for conjugates of proteins with oligosaccharides obtained from the capsular antigens as a result of a partial acidic or enzymic hydrolysis, periodate oxidation, ozonolysis, cleavage with beam of electrons, and these conjugates possess all the necessary epitopes of the parent antigens [23, 52-59].

Liposomes as immunoadjuvants for capsular antigens. Conjugates of bacterial capsular polysaccharides are known to be powerful antigens; however, a substantial disadvantage of these vaccines containing protein can be hypersensitivity and, in some cases, immune response to

the conjugated protein carrier can inhibit response against antigenic determinants of the polysaccharide. In these cases, liposomes possessing comparatively low immunogenicity are suggested for use as an alternative to protein for conjugation with polysaccharides or oligosaccharides in preparation of vaccines [60, 61]. Liposomes are comparatively safe as allergens due to their inertness. Liposomal membranes consisted of phospholipids, cholesterol, and introduced glycolipids and glycoproteins widely used in immunology for various purposes, in particular, for studies of reception, antigenicity, and immunomodulation of polysaccharides and glycoconjugates.

Oligosaccharides representing antigenic determinants of the capsular antigens are the most suitable for preparation of vaccines against encapsulated bacteria using liposomes because they are easily synthesized in a chemical way providing substantial advantages in relation to purity and control of preparation quality. The peak of protective antibodies against polysaccharide is observed 5-7 days after a single intravenous immunization with liposomes containing a complex of oligosaccharide with stearylamine. Immune response against such liposomes as well as against capsular polysaccharides is known to be thymus-independent and deprived of immunological memory. Therefore, such vaccines will be poorly effective for children, especially for infants. These problems can be solved using suitable adjuvant. Thus, e.g., involving into liposome such B-cell mitogens as lipid A (toxic and mitogenic constituent of O-somatic antigens of Gram-negative bacteria) not only stimulates immune response, but also changes immunogenic nature of liposomes in order to induce immunity in newborns. Another group of adjuvants is nonionic block-polymer surfactants as surface-active substances, which stimulate immune response against the conjugates of the capsular antigens with liposomes. The copolymers of polyhydroxyethylene (PHE) with polyhydroxypropylene with the various chain length and average 10% PHE are used as surface-active substances. Immunogenic preparations are obtained using a conjugation of oligosaccharides as immunodeterminants of the capsular antigens with liposomes consisting of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanolamine, cholesterol, and lipid A in molar ratio 40 : 10 : 50 : 1. As a spacer, 2-(4-isothiocyanatophenyl)ethylamine was used. Liposomes prepared in advance are treated with oligosaccharide and spacer for 48 h at room temperature in PBS (phosphate-buffered saline, pH 9). The conjugate obtained after completeness of reaction is dialyzed up to complete removal of excess of oligosaccharide with a spacer and kept at 4°C. The conjugate of liposomes with oligosaccharide can be stored for a long time (one year) without loss of immunogenicity. Before immunization, the conjugate is treated thrice with ultrasound for 30 sec.

When using low doses of conjugate for intraperitoneal immunization of mice, good effect is observed on preliminary injection of surface-active substance (10 nmol) one day before immunization.

At higher doses of the conjugate, immune response is observed also in mouse progeny. It is noteworthy that weaker immune response to the conjugate is observed for male mice in comparison with females. In the presence of surface-active substances, immune response of males is enhanced up to the level of immune response of females immunized without adjuvant but, in spite of that, the protective effect in male remains 60-80% lower than that of females.

In any case, immunization of mice with the conjugate of liposomes with oligosaccharide obtained from the capsular antigen satisfactorily protects from a lethal dose of the corresponding pathogenic microorganism. The immune response possesses some memory: enhanced level of antibodies is observed not only in peak of response, but also substantially later. However, an appreciable protective effect in females is observed for six weeks only after immunization, while the protective effect in males is very low at all times. It should be noted that the conjugates with proteins and peptides cause permanent protection from infection and, therefore, are better immunogens than the conjugates with liposomes, and only in a few cases without adjuvants mice show equally high immune responses for conjugates with proteins and with liposomes. Such behavior is due to the fact that conjugates of oligosaccharides with liposomes fail to involve into immune response T-cells, which are very important for generation of antibodies and immunological memory [61].

Nevertheless, the conjugates of the capsular antigens and their fragments with liposomes can be successfully used in studies of the immune response mechanism. In this case, involving T-cells may be achieved by introduction of epitopes stimulated T-cells in liposomes. Variations in the liposome compositions provide substantial possibilities for investigations of the effects of different factors on immunization with the conjugates of the capsular antigens with liposomes as vaccines [60, 61].

In conclusion, it should be noted that diseases connected directly with capsular polysaccharides are widespread and very severe, especially for infants and the elderly. Considering this, clinical medicine is very interested in development of effective vaccines based on K-antigens against such diseases. The main achievements are related with enhancing effectiveness of vaccines by conjugation with heterologous proteins and peptides and also with use of fragments of polysaccharides and oligosaccharides preserving the main epitopes. In many cases, application of vaccines has led to inducing immunity against diseases caused by encapsulated bacteria and to stable defense of humans and animals against these pathogenic and virulent microorganisms [62]. At present, a sufficiently great number of these vaccines are used in medical practice

[62]. Nevertheless, there are many problems which must be solved. First of all, it is necessary to elucidate a nature of interrelation of capsular polysaccharide structures and their immunomodulatory activity with the goal of influencing the type of immune response by changing structural patterns of the capsular antigens and their conjugates. The capsular antigens are characterized by high variability of immunological activity [62] due to the diversity of their structures.

A particular problem is connected with homology of bacterial capsular polysaccharide structures and their analogs localized on cell membranes of animal cells leading to the absence of immune response to such capsular antigens and sometimes to induction of autoimmunity [63]. The capsular polysaccharides are known to be often haptens and possess poor immunogenicity due to their independence from T-lymphocytes. In some cases a loss of T-lymphocyte memory is observed [64]. In addition, infants and the elderly react weakly to the capsular antigens, causing low efficacy of the corresponding vaccines [65]. Overcoming such and other similar problems will extend the sphere of application of vaccines against various infections and will provide the opportunity to determine the mechanisms of physiologic action of the capsular antigens. Studies on structural features of capsular polysaccharides in connection with their activity are at present a very important problem of researchers of different specialties.

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REFERENCES

1. Jennings, H. J. (1983) *Adv. Carbohydr. Chem. Biochem.*, **41**, 155-208.
2. Cortes, G., Borrell, N., de Astorza, B., Gomez, C., Sauleda, J., and Alberti, S. (2002) *Infect. Immun.*, **70**, 2583-2590.
3. Lee, C.-J. (1987) *Mol. Immunol.*, **24**, 1005-1019.
4. Heidelberger, M. (1973) *Res. Immunochem. Immunobiol.*, **3**, 1-40.
5. Alonso de Velasco, E., Mercus, D., Anderton, S., Verheul, A. F. M., Lizzio, E. F., van der Zee, R., van Eden, W., Hoffman, T., Verhoef, J., and Snippe, H. (1995) *Infect. Immun.*, **63**, 961-968.
6. Roberts, I. S. (1995) *Microbiology*, **141**, 2023-2031.
7. Tallersrud, T., Zernichow, L., Andersen, S. R., Kenny, K., and Lund, L. (2001) *Vaccine*, **19**, 3896-3903.
8. O'Riordan, K., and Lee, J. C. (2004) *Clin. Microbiol. Rev.*, **17**, 218-240.
9. Tzianabos, A. O., Wang, J. Y., and Lee, J. C. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 9365-9370.
10. Heidelberger, M., and Avery, O. T. (1923) *J. Exp. Med.*, **38**, 73-79.
11. Heidelberger, M., and Kendall, F. E. (1935) *J. Exp. Med.*, **61**, 563-591.
12. Heidelberger, M. (1977) *Annu. Rev. Microbiol.*, **31**, 1-12.
13. Francis, J. T., and Tillet, W. S. (1930) *J. Exp. Med.*, **52**, 573-585.
14. Heidelberger, M., Dilapi, M. M., Siegel, M., and Walter, A. W. (1950) *J. Immunol.*, **65**, 535-541.
15. Austrian, R. (1981) *Rev. Infect. Dis.*, Suppl., **3**, s1-s17.
16. Dagan, R., Kayhty, H., Wuorimaa, T., Yaich, M., Bailleux, F., Zamir, O., and Escola, J. (2004) *Pediatr. Infect. Dis. J.*, **23**, 91-98.
17. Kamboj, K. K., Kirchner, H. L., Kimmel, R., Greenspan, N. S., and Schreiber, J. R. (2003) *J. Infect. Dis.*, **187**, 1629-1639.
18. Soininen, A., Pursianen, H., Kilpi, T., and Kayhty, H. (2001) *J. Infect. Dis.*, **184**, 569-576.
19. Misaki, A., Azuma, I., and Yamamura, Y. (1977) *J. Biochem.* (Tokyo), **82**, 1759-1770.
20. Moreau, M., and Schulz, D. (2000) *J. Carbohydr. Chem.*, **19**, 419-434.
21. Goncalves, V. M. M., Tokaji, M., Lima, R. B., Massaldi, H., Giordano, R. C., and Tanizaki, M. M. (2003) *Biotechnol. Appl. Biochem.*, **37**, 283-287.
22. Goncalves, V. M. M., Zangirolami, T. C., Giordano, R. L. C., Raw, I., Tanizaki, M. M., and Giordano, R. C. (2002) *Appl. Microbiol. Biotechnol.*, **59**, 713-717.
23. Pawlowski, A., and Svenson, S. B. (1999) *FEMS Microbiol. Lett.*, **174**, 255-263.
24. Burns, T., Zhong, Z. J., Steinitz, M., and Piforski, L. A. (2003) *Infect. Immun.*, **71**, 6775-6783.
25. Van der Pol, L., Vidarsson, G., Vile, H. A., Vande Winkel, J. G. J., and Rodriguez, M. E. (2000) *J. Infect. Dis.*, **182**, 1139-1145.
26. Shen, X. Z., Lajergard, T., Yang, Y. H., Lindblad, M., Fredriksson, M., and Holngren, J. (2000) *Infect. Immun.*, **68**, 5749-5755.
27. Musher, D. M., Phan, H. M., Watson, D. A., and Baughn, R. E. (2000) *J. Infect. Dis.*, **182**, 158-167.
28. Finn, A., Zhang, Q. B., Seymour, L., Fasching, C., Pettit, E., and Janoff, E. N. (2002) *J. Infect. Dis.*, **186**, 1422-1429.
29. Colino, J., and Outschoorn, I. (2001) *J. Infect. Dis.*, **184**, 1538-1547.
30. Berry, D. S., Lynn, F., Lee, C. H., Frasch, C. E., and Bash, M. C. (2002) *Infect. Immun.*, **70**, 3707-3713.
31. Kenne, L., and Lindberg, B. (1983) *Bacterial Polysaccharides*, in *The Polysaccharides* (Aspinall, G. O., ed.) Vol. 2, Academic Press, N. Y., pp. 314-344.
32. Anderson, P., Ingram, D. L., Pichichero, M. E., and Peter, G. A. (2000) *Pediatr. Infect. Dis. J.*, **19**, 579-591.

33. Herceg, A. (1997) *Commun. Dis. Intell.*, **21**, 173-176.
34. Booy, R., Health, P. T., Slack, M. P. E., Begg, N., and Moxon, E. R. (1997) *Lancet*, **349**, 1197-1202.
35. Porro, M., Costantino, P., Giovannoni, F., Pellegrini, V., Tagliaferri, L., Vanozzi, F., and Viti, S. (1986) *Molec. Immunol.*, **23**, 385-391.
36. Peltola, H. (1998) *Drugs*, **55**, 347-366.
37. Kasper, D. L., Baker, C. J., Baltimore, R. S., Grabb, J. H., Schiffinan, G., and Jennings, H. J. (1979) *J. Exp. Med.*, **149**, 327-339.
38. Mond, J. J., Vos, Q., Lees, A., and Shapper, G. M. (1995) *Curr. Opin. Immunol.*, **7**, 349-354.
39. Mond, J. J., Lees, A., and Shapper, G. M. (1995) *Annu. Rev. Immunol.*, **13**, 655-692.
40. Goebel, W. F., and Avery, O. T. (1931) *J. Exp. Med.*, **54**, 431-436.
41. Avery, O. T., and Goebel, W. F. (1931) *J. Exp. Med.*, **54**, 437-447.
42. Reynaud-Rondier, L., Violand, A., and Michel, G. (1991) *FEMS Microbiol. Immunol.*, **76**, 193-200.
43. Baker, C. J., Paolletti, L. C., Rench, M. A., Guttormsen, H. K., Carey, V. J., Hickman, B. E., and Kasper, D. L. (2000) *J. Infect. Dis.*, **182**, 1129-1138.
44. Alonso de Velasco, E., Verheul, A. F. M., Verhoef, J., and Snippe, H. (1995) *Microbiol. Rev.*, **59**, 591-603.
45. Hu, Y., and Test, S. T. (2004) *Vaccine*, **23**, 21-28.
46. Robbins, J. B., and Schneerson, R. (1990) *J. Infect. Dis.*, **161**, 821-832.
47. Paradiso, P. R., Dermody, K., and Pillai, S. (1993) *Vaccine Res.*, **2**, 239-248.
48. Huo, Z., Spencer, O., Miles, J., Johnson, J., Holliman, R., Sheldon, J., and Riches, P. (2004) *Vaccine*, **22**, 1157-1161.
49. Korneila, M., Lehtonen, H., Ahman, H., Leroy, O., Eskola, J., and Kayhty, H. (2000) *Vaccine*, **18**, 1217-1226.
50. Waite, E. R., and March, J. B. (2002) *J. Comp. Pathol.*, **126**, 171-182.
51. Alexander, J., del Guercio, M. F., Frame, B., Maewal, A., Sette, A., Nahm, M. H., and Newman, M. J. (2004) *Vaccine*, **22**, 2362-2367.
52. Zou, W., Laferriere, C. A., and Jennings, H. J. (1998) *Carbohydr. Res.*, **309**, 297-301.
53. Lefeber, D. J., Gallego, R. G., Grun, C. H., Proietti, D., D'Ascenzi, S., Costantino, P., Kamerling, J. P., and Vliegenthart, J. F. G. (2002) *Carbohydr. Res.*, **337**, 819-825.
54. Lefeber, D. J., Arevalo, A., Kamerling, J. P., and Vliegenthart, J. F. G. (2002) *Can. J. Chem.*, **80**, 76-81.
55. Laferriere, G. A., Sood, R. M., de Muys, J.-M., Michon, F., and Jennings, H. (1997) *Vaccine*, **15**, 179-186.
56. Beuvery, E. C., van Rossum, F., and Nagel, J. (1982) *Infect. Immun.*, **37**, 15-22.
57. Svenson, S. B., and Lindberg, A. A. (1977) *FEMS Microbiol. Lett.*, **1**, 145-148.
58. Anderson, P. W., Pichichero, M. E., Insel, R. A., Betts, R., Ebyi, R., and Smith, D. H. (1986) *J. Immunol.*, **137**, 1181-1186.
59. Wang, Y., Hallingsworth, R. I., and Kasper, D. (1998) *Proc. Natl. Acad. Sci. USA*, **84**, 9170-9174.
60. Zigterman, G. J. W. J., Verheul, A. F. M., and Snippe, H. (1997) in *Drug Delivery*, Vol. 2, *Liposomes in Drug Delivery* (Gregoriadis, G., Flores, A. T., and Pavel, H. M., eds.) Chap. 5, pp. 67-76.
61. Burgeot, C., Gilbert, F. B., and Poutrel, B. (2001) *Vaccine*, **19**, 2092-2099.
62. Weintraub, A. (2003) *Carbohydr. Res.*, **338**, 2539-2547.
63. Finne, J., Bitter-Snermann, D., Goridis, C., and Finne, U. (1987) *J. Immunol.*, **138**, 4402-4407.
64. Howard, J. (1987) in *Towards Better Carbohydrate Vaccines* (Bell, R., and Torrigiani, T., eds.) J. Wiley and Sons, Chichester, UK, pp. 221-232.
65. Bondada, S., Wu, H.-J., Robertson, D. A., and Chelvarajan, R. L. (2001) *Vaccine*, **19**, 557-565.