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Review

Immunogenicity and immunochemistry of *Streptococcus pneumoniae* capsular polysaccharides

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I. Introduction

Infections with *Streptococcus pneumoniae* continue to cause significant morbidity and mortality in humans, despite the availability of effective antibiotic therapy and of a multispecific capsular polysaccharide vaccine (Austrian 1981). The most common infections caused by pneumococci are otitis media, pneumonia (often accompanied by bacteremia), meningitis, and septicemia with or without a focus. Death often occurs before antibiotic therapy can influence the course of the disease. Moreover, there is concern about a world-wide development of antibiotic-resistant pneumococci (Applebaum 1987; Pallares et al. 1987). These facts have prompted renewed efforts to prevent pneumococcal disease by vaccination.

Of the 85 known capsular types (Kauffman et al. 1960, Tables 1 and 2) some are especially virulent in humans and others are not. The composition of the current 23 valent capsular polysaccharide vaccine (Pneumovax©) is based on the isolated serotypes responsible for ~ 90% of the infections in the U.S. (Robbins et al. 1983, Table 1). However, the types most frequently isolated may vary from year to year and with the geographical area (Finland & Barnes 1977; Moulin et al. 1979; Dixon & Lipinsky 1981), which may necessitate adaptations of the composition of the vaccine. Striking differences in case fatality rates among pneumococcal types have been observed ranging from an 8% mortality rate among patients infected with type 1, to a 55% rate with type 3 (Austrian & Gold 1964; Mufson et al. 1974). Although several types appear to have a special predilection for immunocompromized patients (Weisholz et al. 1983), there is no obvious association of a particular type with any clinical condition (Mufson et al. 1982).

Capsular polysaccharides generally are poor immunogens, since they elicit only weak primary antibody responses and give no memory response. In mice polysaccharide antigens readily initiate a state of specific tolerance. In healthy adults a significant increase in specific antibody titres is observed after vaccination, which then slowly decline (Leinonen 1982).

S. pneumoniae forms part of the normal flora of the respiratory tract, and as long as the host has a competent immune system a peaceful coexistence may be maintained. Pneumococcal pneumonia is rarely a primary infection of the lung, but factors as damage to the respiratory tract, fatigue, chilling of the body and general debilitation predispose to infection. The high-risk groups are elderly persons and infants, and patients with chronic debilitating conditions such as disorders of the respiratory tract, of metabolism and of the reticuloendothelial system, including the immune system (Lipsky et al. 1986; Gruer et al. 1984). The immune response to the pneumococcal polysaccharide vaccine in the population at greatest risk has frequently been reported to be impaired (Musher et al. 1986) and some cases of failure of the vaccine have cast doubt on its efficacy (Zarrabi & Rosner 1985; Simberkoff et al. 1986; Williams 1987). In elderly persons prevaccination antibody levels are generally lower but

increase several fold after vaccination. In infants under 6 months, to a great number of polysaccharides no antibodies are developed. From the age of 6 months to 2 years the response is increasing, but the response to certain pneumococcal types (e.g. types 6AB, 14, 19F, and 23F) remains poor, and antibody titres return rapidly to prevaccination levels (Leinonen 1982). As high-risk patients have impaired antibody responses, the duration of elevated antibody levels may be shorter than in healthy controls. Alternative strategies are needed to maximize the protective effect of the pneumococcal vaccine. Design of improved vaccines to *S. pneumoniae* requires insight into the pathogenic potential of the pneumococcus and its interaction with host defense mechanisms, i.e. into the pathogenesis of pneumococcal disease. Moreover, information is needed on the functional effectiveness of antibodies induced by various vaccines, i.e. opsonic activity and efficiency in promoting leukocyte-mediated killing.

In this review efforts are made to find the relations of chemical structures with biological responses, in order to understand in more detail the mechanisms of virulence and to reach to the design of an effective pneumococcal vaccine.

II. Immunological defense mechanisms

The immunological defense mechanisms which resist invasion by *S. pneumoniae* are complex interactions between various host cells, humoral components and the bacteria. Encapsulated pneumococci resist the non-specific host defense mechanism of lysozyme digestion and direct adhesion to macrophages and granulocytes, although lysozyme induces growth-inhibitory effects on type 1 pneumococci in vitro (Jacquot et al. 1987). Fever also markedly slows growth-rates, but does not prevent development of meningitis in rabbits (Sande et al. 1987). The factors that are important for elimination of invading pneumococci are phagocytosis by macrophages or granulocytes, for which activation of the complement system and the production of specific antibodies is a prerequisite.

Skin and mucous membranes – adhesion

The skin and mucous membranes constitute the first barrier against bacterial invasion. The first step in pneumococcal disease necessarily requires adhesion of the bacteria to the host's mucosal membranes, in the case of pneumococci primarily the nasopharynx. Only after extending to other areas of the respiratory tract or after penetration of the nasopharyngeal mucosa to reach the systemic circulation via the cervical lymphatics, does it give rise to disease (Austrian 1986). Under less favourable conditions the organisms become avirulent, lose their capsule and their immunological specificity.

Attaching pneumococci specifically bind to glycoconjugate receptors on the collagen-binding region of fibronectin, present on the surface of human pharyngeal epithelial cells. These receptors contain the disaccharide β-D-GlcNAc*p*(1→3)-β-D-Gal*p*, which (substituted at various positions) is part of many saccharide chains active as blood-group determinants (ABH, Lewis and I/i antigens) (Andersson et al. 1983a). Non-secretion of blood-group substances (ABH antigens) predisposes to infections (Blackwell et al. 1986). Lungs and meninges are closely settled with bacteria, whereas attachment to other organs (e.g. liver, spleen, brain) is lacking. Blocking of bacterial lectins by GlcNAc prevents adherence in vitro of *S. pneumoniae* (Beuth et al. 1987), and significantly decreased attachment of pneumococci to epithelial cells is observed when the bacteria are preincubated with lactosamine-oligosaccharides from human milk (Andersson et al. 1985). Also the casein fraction of human milk, which contains these carbohydrate structures, inhibits attachment of pneumococci (Aniansson et al. 1988). The adhesin secreted by pneumococci, which is possibly related to the competence factor, required to pneumococci for exogenous DNA reception and transformation, forms a bridge between the bacterial surface and a receptor on the epithelial cells (Andersson et al. 1988). Adherence of type 1 pneumococci to the tracheal epithelium of mice is enhanced by prior influenza virus infection; so viral respiratory infections may play a

Table 1. Streptococcus pneumoniae capsular types, *A*, Danish and *B*, American nomenclature, *C*, polysaccharide vaccine formulation (Pneumovax© 14 and 23), and *D*, distribution of types and types within groups isolated from blood or CSF (Robbins et al. 1983).

A*	B	C		D		A	B	C		D	
		14	23	%	%			14	23	%	%
1	1	+	+	7.5		20	20	–	+	1.4	
2	2	+	+	0.9		21	21	–	–		
3	3	+	+	6.9		22F	22	–	+	1.6	99
4	4	+	+	8.3		22A	63	–	–		1
5	5	–	+	2.1		23F	23	+	+	5.3	93
6A	6	+	–		39	23A	46	–	–		4
6B	26	–	+	7.4	61	23B	64	–	–		4
7F	51	+	+	5.7	96	24F	24	–	–	0.6	
7A	7	–	–			24A	65	–	–		
7B	48	–	–			24B	60?	–	–		
7C	50	–	–		4	25F	25	+	–	0.8	
8	8	+	+	6.0		25A	–	–	–		
9A	33	–	–		8	27	27	–	–		
9L	49	–	–		2	28F	28	–	–		
9N	9	+	+	2.2	57	28A	79	–	–		
9V	68	–	+	3.2	34	29	29	–	–		
10F	10	–	–		11	31	31	–	–	0.5	
10A	34	–	+	1.0	89	32F	32	–	–		
11F	11	–	–			32A	67	–	–		
11A	43	–	+		78	33F	70	–	+	1.2	79
11B	76	–	–	1.0	17	33A	40	–	–		14
11C	53	–	–		5	33B	42	–	–		3
12F	12	+	+	3.0	84	33C	39	–	–		4
12A	–	–	–		16	34	41	–	–		
13	13	–	–	0.7		35A	47,62	–	–		
14	14	+	+	9.4		35F	35	–	–		
15F	15	–	–		9	35B	66	–	–		
15A	30	–	–		31	35C	61	–	–		
15B	54	–	+	1.0	22	36	36	–	–		
15C	77	–	–		39	37	37	–	–		
16F	16	–	–	0.8		38	71	–	–		
16A	–	–	–			39	69	–	–		
17F	17	–	+	0.9	88	40	45	–	–		
17A	78	–	–		12	41F	38	–	–		
18F	18	–	–		3	41A	74	–	–		
18A	44	–	–		4	42	80	–	–		
18B	55	–	–		10	43	75	–	–		
18C	56	+	+	5.2	83	44	81	–	–		
19F	19	+	+	4.5	65	45	72	–	–		
19A	57	–	+	2.5	34	46	73	–	–		
19B	58	–	–		1	47F	52	–	–		
19C	59	–	–			47A	–	–	–		
						48	–	–	–		

* Now generally used.

role in the pathogenesis of pneumonia (Plotkowski et al. 1986). Viral neuraminidase might reveal cellular receptors for pneumococcal adhesion.

The adhesive capacity is not determined by the capsular type, but stronger adhesion has been observed for strains isolated from the nasopharynx of patients with otitis media, than when isolated from blood or cerebrospinal fluid (Andersson et al. 1983b).

Pneumolysin, a sulfhydryl-activated cytolysin produced by pneumococci, has a toxic effect on epithelial cells. It is not secreted by the bacterium, but is released during autolysis. Release of pneumolysin in the respiratory tract during infection may perturb host defences, allowing bacterial proliferation and spread (Steinford et al. 1989).

In rat alveolar lining material, the presence of long-chain free fatty acids and other soluble factors, which are bactericidal for pneumococci in vitro, has been demonstrated (Coonrod et al. 1987a). Early extracellular killing of inhaled pneumococci occurs by an autolysin independent mechanism, and is associated with increased permeability of the pneumococcal cell membrane.

Once the microorganisms have breached the mucocutaneous barrier, concerted action by phagocytes and opsonins, i.e. antibodies and complement, is required to clear the bacteria from tissue sites and the bloodstream.

Response of the immune system

Surface components of the bacteria are implicated in interactions with the immune system. Capsular antigens are responsible both for the stimulation of the immune response and for the virulence of the encapsulated bacteria. For example *S. pneumoniae* type 3, one of the commonest isolates, is remarkably resistant to phagocytosis, and yet potently immunogenic. In contrast, group 6 pneumococci, frequent pathogens in childhood, are readily phagocytized, but have a capsule which is a much less effective antigen.

1. Phagocytes

Survival of the host requires removal of the organisms from the blood stream by cells that can ingest and destroy them. These cells include circulating polymorphonuclear neutrophil leukocytes (PMN's) and monocytes, as well as splenic macrophages and hepatic Kupffer cells (Rogers 1960).

Generally, capsular polysaccharides are highly polar and hydrophilic and interfere with cell-to-cell interactions with phagocytes. The ability of host phagocytes to ingest and kill invading organisms such as encapsulated pneumococci requires coating of the bacteria by antibodies and complement, a process called opsonization. Antibodies bound to capsules, negating charge and hydrophilicity, may act as bacterial cell-to-phagocytic cell ligands or as complement-activator. Although opsonization by antibodies and complement appears to be essential in host defense against encapsulated bacteria, evidence has been presented that under certain conditions both PMN's and alveolar macrophages can use an opsonin-independent mechanism of phagocytosis when they encounter bacteria adhering to a surface (Lee et al. 1984). Lectinophagocytosis may be mediated by lectins on bacterial surfaces, which recognize sugar residues on the surfaces of phagocytes, or phagocytic surface lectins, which recognize sugar residues of the bacteria (Ofek & Sharon 1988). However, there is extensive evidence that, for effective clearance of bacteria by liver and spleen, antibodies and complement are an absolute requirement. In vitro opsonophagocytosis of type 1 pneumococci did neither correlate with antibody concentration nor with mouse protection, whereas with type 3 phagocytic killing could be correlated with anti-S3 antibody concentrations (Fine et al. 1988).

PMN's accumulate in large numbers at the site of infection (alveoli) in the early inflammatory process (Lichter et al. 1984). PMN recruitment is mediated by intra-alveolar chemotaxins (Vial et al. 1984). Growing pneumococci liberate factors chemotactic for PMN (Ward et al. 1968). In addition, complement components (C3a, C5a) (Toews & Vial 1984) and chemotactic metabolites of the arachidonic acid pathway (lipoxygenase products like

leukotriene B_4) (Tuomanen et al. 1986), released by alveolar macrophages or cerebrospinal fluid monocytes during phagocytosis, play a role in PMN recruitment. Pneumococcal challenge of the lung induces both granulocytopenia and pulmonary leukostasis in normal animals, while complement-depleted animals display granulocytopenia without pulmonary leukostasis (Goldblum et al. 1983). Poor pulmonary granulocyte recruitment has been observed in newborn rats (Coonrod et al. 1987b). Pneumococcal constituents (MW 100–300kD, not the capsular polysaccharide) are involved in the induction of granulocytopenia. Complement depletion gives a more than hundredfold increase in LD_{50} for types 2 and 19F pneumococci, which seems to be related to diminished efficiency of leukocyte-mediated killing, while cerebrospinal fluid leukocytosis and the rate of clearance are not affected (Tuomanen et al. 1986).

Considerable heterogeneity exists among encapsulated pneumococci in their resistance to granulocyte killing. The degree of resistance to phagocytosis and killing by granulocytes is not consistent with the pathogenicity of the serotypes (Braconier & Odeberg 1982). Types 31 and 36, which rarely cause infections in humans, are as resistant as commonly recovered types 3, 6 or 19, while types 14, 23, 35, 42, and 43 are less resistant.

Pre-incubation of human serum with *S. pneumoniae* type 1, 12F, and 25F capsular polysaccharides (S1, S12F, and S25F) at 0° did not affect the capacity of this serum to induce leukocyte-mediated killing of the various pneumococcal types (Schweinle 1986). When the incubation time with S1 was increased, a decline in killing of type 25F pneumococci was observed, but less impairment in killing of type 12F and no impairment for type 1. After prolonged incubation with S25F, human serum did not support killing of type 25F, while normal killing of types 1 and 12F was observed. Incubation with S12F impairs killing of types 12F and 25F but not of type 1. Addition of specific antisera did not restore killing. These data suggest that preincubation of human serum with some capsular polysaccharides may result in depletion of essential opsonins other than specific anti-capsular antibodies.

Purified pneumolysin inhibits the bactericidal activity of human PMN's and macrophages, and pneumolysin-negative mutants show reduced virulence for mice (Berry et al. 1989). Although the in vitro growth rates are identical, there are differences in clearance from the blood with normal strains, which may be caused by differences in the rate of in vivo multiplication and/or more efficient phagocytic clearance.

2. Antibodies

There are three patterns of antibody-mediated opsonization for pneumococci. Specific antibodies can act as opsonins by attaching directly to immunoglobulin G (IgG) Fc receptors on the phagocyte surface. This mechanism is important only in a hyperimmune state, when intense interaction has taken place, resulting in specific antibody-producing B-lymphocytes. Opsonization may also occur by type-specific antibodies, acting in concert with the classical complement pathway, or by 'non-specifically' adhering immunoglobulin molecules and the alternative pathway. The main role of antibodies in the opsonization of *S. pneumoniae* is to mediate fixation of the opsonic complement component C3b to the bacterial surface through activation of the classical complement pathway. Anti-capsular antibodies increase the rate of clearance of pneumococci, primarily via activation of the classical complement pathway. Although, in general, clearance of bacteria from the bloodstream occurs mainly through the liver and spleen, it has been shown that anti-capsular antibodies increase hepatic clearance and decrease uptake by the spleen (Brown et al. 1983a).

2.1. Lymphocytes (antibody production)

Antibody production to most antigens is the final result from interactions of macrophages (antigen presenting cells), thymus-derived (T-cells) and bone-marrow-derived lymphocytes (B-cells). B-Cells develop specificity for antigens during ontogeny and produce specific antibodies when they become mature plasma cells, after stimulation by antigen and T-cells (T-helper, T_H-cells). These types of antigens are called thymus dependent

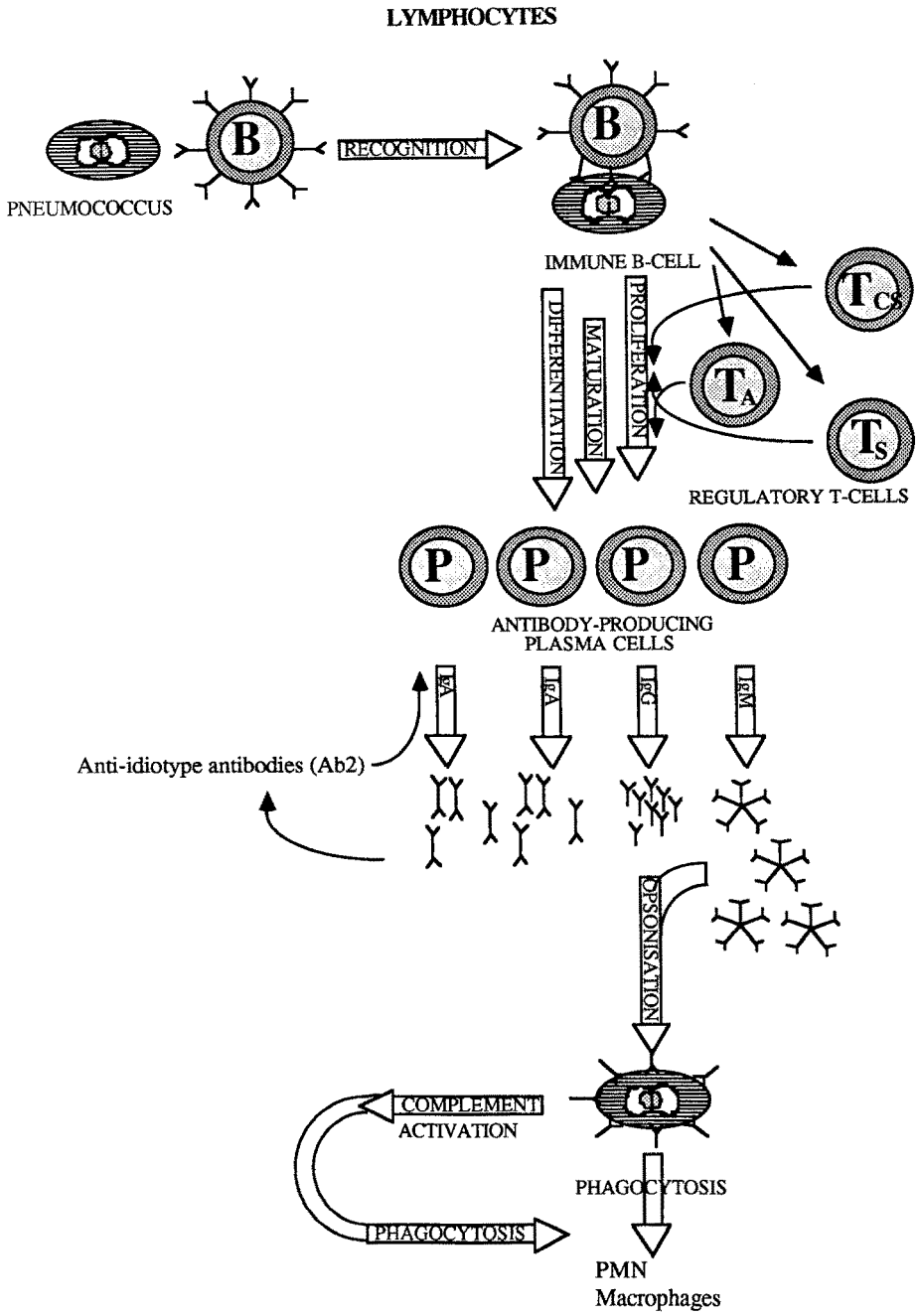
(TD). There are, however, a number of antigens (among which polysaccharide antigens), which are capable of activating B-cells to produce antibodies independently of T-cell help, and are therefore referred to as thymus independent antigens (TI). Most TI-antigens are resistant to degradation and are taken up in the marginal zone macrophages in the lymphoid organs. In vitro a definitive role for macrophages as antigen presenting cells has been demonstrated (Ada 1987), but their role in B-cell activation by polysaccharides in vivo remains to be clarified. The magnitude of immunoglobulin M (IgM), but also IgG and IgA antibody response to polysaccharide antigens (which is studied most extensively for S3) is governed by the activities of regulatory T-cells (T-amplifier T_A, T-suppressor T_S, and T-contrasuppressor T_{CS}; Scheme 1) (Barthold et al. 1974).

2.1.1. B-cells. Polysaccharide binding lymphoid cells (large B-cells), and polysaccharide specific plaque forming cells appear and disappear rapidly from the circulation after immunization. In mice polysaccharide-specific plasma cells appear 3 days after immunization in the circulation, predominantly in the spleen, but later on (day >20) also in other lymphoid tissues (e.g. bone marrow). These proliferating mononuclear cells synthesize large amounts of polysaccharide-specific IgM antibodies. This process is modified by T-cells, T-cell factors or antigen (Kehrl & Fauci 1983a).

In humans anti-polysaccharide-secreting plasma-cells are present for a limited period between postvaccination days 3 and 13, with a maximum of specific antibody-secreting cells, constituting 20–80% of the total antibody-secreting cells, between days 6 and 9 (Heilmann & Pedersen 1986; Kehrl & Fauci 1983b). Together with the increase in antibody-secreting cells, increasing numbers of polyclonally activated antibody-secreting cells are present. The majority of polysaccharide antigen-binding cells contain membrane-bound IgG (Kehrl & Fauci 1983c). After vaccination of healthy volunteers with Pneumovax© 23, the total number of B-cells secreting specific IgM antibodies in blood reach a maximum at day 6 ($552/10^6$ mononuclear cells), while IgG and IgA-secreting cells have the highest concentrations at day 7 (628 and $1691/10^6$ mononuclear cells, respectively) (Heilmann et al. 1987). The predominance of IgA antibody-secreting cells is explained by the greater tendency of these cells to enter the circulation.

B-Cells secreting antibodies to S3, S8, S18C and C-polysaccharide constitute 9, 16, 6, and 5% of the total number of antibody-secreting cells, respectively. Anti-C polysaccharide-secreting cells almost exclusively secrete IgG, which also are present before vaccination in high concentrations (Heilmann et al. 1987). Antigen-dose influences the idiotypy of the primary anti-C response (Stout et al. 1985).

2.1.2. Regulatory T-cells. Human polysaccharide-specific B-cells are activated in the absence of T-cells, but they may augment the B-cell response (Rijkers & Moshier 1985). T_S-cells act primarily by limiting the extent to which bone-marrow-derived precursors of B-cells proliferate following immunization (Baker et al. 1974a; Heilmann 1987). The action of T_S-cells is well characterized for S3, but is not generally applicable to all polysaccharides. Immune B-cells provide a signal or stimulus for the activation of T_S-cells, which regulate the magnitude of the antibody response by limiting the extent to which antigen-stimulated B-cells proliferate in response to S3. Low (subimmunogenic) doses of S3 result in the development of an antigen specific unresponsive state (low-dose paralysis), which is a T-cell-dependent phenomenon in which T_S-cells activated by low doses of antigen play a major role (Baker et al. 1974b). T_S-cells are activated in response to the idiotypic determinants of B-cell-associated antibody (Taylor et al. 1983) specific for S3. T_S-cells generated in response to S3 function by releasing a soluble factor (or factors) that bind(s) to determinants on B-cells rather than antigen (Taylor & Baker 1985). This factor acts directly on antigen-stimulated B-cells or inhibits the induction of T_A-cells. T_A-cells can be induced and activated by exposure to immune B-cells, with a specificity due to the ability of T_A-cells to recognize the idiotypic determinants determinants of B-cell-associated antibody specific for S3 (Taylor et al. 1984a). Platelet factor 4 interferes with induction of S14-



Scheme 1. Diagrammatic representation of the lymphocyte response to encapsulated bacteria, resulting in the production of antibodies and phagocytosis.

specific T_S-cells, but is less effective in reversing low dose tolerance to S3, suggesting different mechanisms of tolerance induction for S3 and S14 (Yin et al. 1988).

The antibody response to S3 is enhanced by some lectins like concanavalin A (Con A) and phytohemagglutinin (PHA), which might activate subsets of regulatory T-lymphocytes bearing appropri-

ate receptors specific for that lectin. Con A activates T_S as well as T_A involved in the antibody response to S3, dependent on the time at which the lectin is given relative to S3 (Baker at al. 1981; Markham et al. 1977), while PHA augments T_A-cell activity, regardless of when it is given (Taylor et al. 1984b).

Activation of T_{CS}-cells by immunogenic doses of S3 is required for the induction of an antibody response to S3 (Braley-Mullen 1986a). The interaction of T_{CS}-cells with B-cells or T_S-cells is via an antigen bridge rather than an idiotype-anti-idiotype interaction (Braley-Mullen 1986b).

Natural killer (NK) cells act as regulators of the antibody responses to polysaccharide antigens. The NK cells down-regulate anti-polysaccharide responses, the primary determinant of age-related and type-specific variations in these responses (Khater et al. 1986). Other factors that influence these variations are deficiency or immaturity of specific B-cells, excessive T_S-cell activity, and deficient T_A-cell activity. Neonatal B-cells fail to generate an in vitro antibody response to S4, which cannot be ascribed to functional T-cell immaturity (Rijkers et al. 1987).

2.2. Immunoglobulins (isotypes, subclasses, and functions)

Immunoglobulin molecules are built up from a constant region (Fc), which structure determines the immunoglobulin class or isotype, and a variable region (Fab), which contain the antigen-binding pocket (idiotype or mirror image of the epitope). The antibody response in humans to pneumococcal polysaccharides involves IgG, IgA, and IgM isotypes.

IgA antibodies do not protect against systemic infection with pneumococci, but inhibit infections without causing inflammatory processes by interfering with the adherence of infectious agents to mucosal surfaces. The majority of antigen specific IgA-secreting cells in adults produce antibodies of the IgA2 isotype (Heilmann et al. 1988; Lue et al. 1988). The presence of IgA bound on the surface of mouse lung lymphocytes plays a role in the natural anti-bacterial activity against *S. pneumoniae* (Sestini et al. 1988).

In healthy adults, anti-S4 and anti-S7F IgG antibodies are predominantly of the IgG1 and IgG2 subclasses (Chudwin et al. 1987), which differ in structure (amino acid sequences) and functional properties (activation of complement). Determination of specific anti-capsular polysaccharide IgG and IgA subclasses in healthy individuals show high IgG2 levels in cord blood and in serum from adults, while IgG1 is found primarily in young children (Rynnell-Dagöö et al. 1986) and is less abundant in adults. The allotype G2m(n) of vaccinees influences the levels of anti-S14 and anti-S18C IgG2, but less so in the anti-S3 response (Sarvas et al. 1989). This allotype has no detectable effect on the levels of IgG1, IgG4, or IgM antibodies. Low antibody concentrations are observed in IgG2-deficient donors, but not in IgG3-deficient ones. IgA subclass antipneumococcal activity is also correlated to age. Children over 5 years of age produce IgA antibodies against all types (Leinonen 1982), while children under 2 only occasionally form IgA.

Recent studies on the clonal variation among different populations of B-cells indicated that essentially all IgM- and IgA-secreting cells isolated after vaccination secrete anti-polysaccharide antibodies (Heilmann & Barington 1989), whereas only approximately 60% of the IgG-secreting cells secrete anti-polysaccharide antibodies. Interestingly, the distribution of κ and λ light chain isotypes on these secreting cells revealed that the IgA-secreting cells are derived from B-cell clones with a limited idiotypic heterogeneity, due to selection by natural occuring antigens at the mucosal membranes.

Specific anti-capsular IgG of restricted heterogeneity is readily absorbed in large quantities by type 3 or 8 pneumococci (type 3: 5×10^7 molecules IgG/CFU, type 8: 5×10^6 molecules IgG/CFU). Absorption is dependent on the specific $F(ab')_2$ fragment of the immunoglobulin (Reed et al. 1983), which not only reacts with the surface of the capsule, but also can penetrate and be distributed throughout the capsule. Anti-capsular IgG in the absence of complement does not promote phagocytosis by human or rabbit PMN's, below levels that cause agglutination. In the presence of complement, only 10% of the IgG is needed to promote

phagocytosis (~ 1% of the antibodies that can be absorbed by the organisms), emphasizing the essential role of complement in promoting efficient phagocytosis of pneumococci.

During the primary response induced by an optimal dose of S3, two specific subclasses of IgM antibodies are formed in mice (Igm1 and Igm2) with complement-fixing and non-complement-fixing activities (Kearney & Johnstone 1985). When non-complement-fixing anti-S3 antibodies are present, no high-dose tolerance is induced, and low-dose paralysis in the complement-fixing IgM response to S3 might not be mediated by T_S-cells, but can be attributed to highly avid non-complement-fixing IgM anti-S3-antibodies (Kearney et al. 1986), formed preferentially at low doses of S3. The presence of specific serum anti-capsular antibodies does not necessarily imply immunity to pneumococci.

The IgA anti-S3 activity in serum of S3-immune mice on day 5 of the primary response is due to hybrid IgM/A(κ) antibodies, which are absent in athymic mice (Kearney 1983).

S. pneumoniae produces extracellular human immunoglobulin-specific proteases (Wikström et al. 1984), such as a protease that hydrolyses secretory IgA1 antibodies (Kornfeld & Plaut 1981), which may facilitate both bacterial colonization of mucous membranes, and penetration through the mucosal barrier of the upper respiratory tract. Endo-glycosidases produced by *S. pneumoniae* remove carbohydrates from IgA1 proteins (Kilian et al. 1980). No correlations have been found between the type and the ability to degrade immunoglobulins.

2.3. Tolerance

Inoculation of high doses of most polysaccharides induce tolerance, a state of specific unresponsiveness (paralysis), which is generally a B-cell phenomenon and of very long duration. The incapacity of the immune system to respond to a secondary injection of a polysaccharide antigen is a consequence of its persistence in the organism. Usually, antigens with repetitive epitopes are poorly or non-degradable in vivo (Moreno 1987). Polysaccharides which degrade rather quickly in vitro (and in vivo), like the phosphorylated polysaccharides of types 6AB and 19F which are degraded by alkaline phosphatase, are neither immunogenic nor tolerogenic, since the immunogenicity of polysaccharides is directly related to the molecular mass. Other polysaccharides are not biodegradable, and persist in the tissue for life. Immunogenicity has been restored in tolerant animals injected with glycolytic enzymes specific for S3 (Avery & Dubos 1931; Brooke 1964).

2.4. Memory

Elevated anti-capsular antibody levels are observed 10 years after vaccination in more than 50% of the vaccinees for types 4, 7F, and 8 but not for types 1, 3, 12F, 14, and 19F. Revaccination gives lower raise in anti-capsular antibodies than primary immunization (Mufson et al. 1987).

Polysaccharide antigens, however, are TI-antigens and capable of triggering B-cells to IgM production in mice. In humans the antibodies produced are of IgM, IgG and IgA classes, but the increase in IgG (20%) and IgA (70%) antibodies is generally more pronounced than the increase in IgM (10%) antibodies (Heilman et al. 1987; Pedersen 1983; Pedersen & Henrichsen 1982). The inability of TI-antigens to induce memory IgG-producing B-cells is due to the fact that TI-antigens do not activate T_H-cells. Antigens need to contain domains that interact with T_H-cells and the histocompatibility (MHC) Ia antigens on antigen presenting B-cells for induction of cell-mediated immunity (Guilet et al. 1987). The combination of antigen and (MHC) Ia molecules is transferred to the surface of antigen-presenting cells (often mononuclear phagocytes), where it is presented for further processing by T_H-cells, that recognize the antigen-MHC combination. TI-antigens can interfere with induction (in a dose-dependent way) or expression of B-memory (Wilson & Braley-Mullen 1981), and in mice the antibodies are restricted to the IgM class. Antibody responses to TI-antigens are followed by the production of anti-idiotype antibodies (Ab2), which are down-regulators of subsequent idiotype responses (Taylor et al. 1983). Production of a (non-immunoglobulin) antifactor is found in mice after immunization with S3 (Pasa-

nen 1986). Moreover, the response to TI-antigens are under the negative control of prostaglandins.

Enhanced numbers of S3-specific IgG-producing cells (IgG2a and 2b subclass) have been observed after T_A-cell activation by allogeneic stimulation by PHA at the time of S3 immunization, but no immunologic memory is induced (Busby & Roberson 1981). Predominance of human IgG2 antibody response for types 3, 6A, 18C, 19F, and 23F is analogous to the murine immune response to type 2 TI-immunogens (TI-2), which show IgG subclass restriction, and the requirement of a mature B-cell subset (defined by the $Lyb5^+$ alloantiserum) (Barrett & Ayoub 1986).

Mice produce IgG antibodies specific for S3 when immunized with a T-cell-dependent (TD) form of antigen (S3 coupled to protein carriers or sheep erythrocytes [S3-SRBC]) and develop S3-specific memory (Paul et al. 1971; Braley-Mullen 1974, 1980; Beuvery et al. 1982). Some strains of mice undergoing an allogeneic effect also produce IgG antibodies when immunized with S3. Activation of T-cells by immunization with S3 together with allogeneic spleen cells leads to increased IgM response of mice, which are genetic low responders to S3 or to S3-SRBC. Moreover, it induces IgG antibody formation and S3 immune memory in both high and low responder strains of mice. S3 coupled to syngeneic spleen cells can induce tolerance (Braley-Mullen 1982), regulated by antigen-specific T_S- and T_{CS}-cells, in euthymic but not athymic mice (Braley-Mullen 1984).

Low doses of the TI-2 antigens S3 and S19F activate T_H capable of providing help to B-cells primed with S3 or S19F coupled to erythrocytes for a secondary IgG response (Milligan & Braley-Mullen 1989). Higher doses activate T_S-cells.

Studies with recombinant-inbred strains of mice have indicated that multiple genes (not linked to the major histocompatibility complex (H-2) or the immunoglobulin CH (IgCH) locus of mice) act independently to influence the magnitude of the antibody response to S3 (Baker et al. 1976). Autosomal as well as X-linked genes influence serum IgM levels (Baker et al. 1984).

2.5. Circulating immune complexes

Patients with pneumococcal pneumonia have elevated levels of circulating immune complexes, containing mostly IgG and only little IgM (Mellencamp et al. 1987), which may play a pathogenic role, as they have been associated with the development of glomerulonephritis and pulmonary alveolitis in adults recovering from pneumococcal infections. Serum from non-bacteremic patients contains higher levels of IgG-containing immune complexes than samples from bacteremic patients. Immune complexes contribute to lung damage via complement and neutrophil activation (Rytel & Preheim 1986). In 93% of the cases, they remain present after recovery from the disease and during the follow-up period (Prober et al. 1983). In some of these cases, concentrations of complement components C3 and C4 and factor B are depressed. The failure of natural infection to elicit antibodies may be due to the release of soluble antigen from infected tissue into the circulation. Free antigen may neutralize antibodies by producing immune complexes. Mortality due to pneumococcal disease has been reported to correlate with circulating capsular polysaccharide, rather than bacteremia (Bukantz et al. 1942).

Increased IgA synthesis following antigenic stimulation at mucosal surfaces may lead to IgA glomerulonephritis, resulting from deposition within the kidney of IgA-containing immune complexes (Drew et al. 1987).

3. Complement

Like other Gram-positive bacteria, pneumococci are not killed by the action of antibody and complement. The role of complement is limited to opsonic and chemotactic activities. The serum proteins of the complement system function in an ordered and integrated fashion (Scheme 2) as mediators of inflammation and host defense (Winkelstein 1984). Complement is present in the alveolar lining material, and plays a role in pulmonary defenses, by generation of chemotaxins and the C3 opsonin. Decomplemented rats show increased sensitivity to pneumonia caused by *S. pneumoniae* types 3 and

25F (Coonrod & Yoneda 1982). Both IgG and IgM anti-S7 antibodies activate complement, but IgM is the most efficient complement activator (Brown et al. 1982). IgA does not activate complement and even blocks initiation of complement by antibodies of other isotypes (Griffiss & Goroff 1983). Anti-cell-wall antibodies fail to mediate complement-dependent phagocytosis. Although anti-cell-wall IgG is capable of activating complement and fixing C3 to pneumococci, it is not opsonic.

Interaction of pneumococci with the complement system may vary according to type. For example, type 3 activates the classical complement pathway, while type 25F exclusively activates the alternative complement pathway, and type 14 activates both of them (Cheson et al. 1984). Types 6A, 18F, 19A, 23F and 25F are opsonized efficiently by the alternative pathway (Giebink et al. 1977; Matthay et al. 1981), whereas types 3, 4 and 8 activate complement only in the presence of specific antibodies (Fine 1975).

Comparison of the activation of the two pathways by different strains with their non-immune reactivity with the Fc region of IgG shows that highly Fc-reactive strains (e.g. types 33, 5, 19, 29, 20 and 10)* activate the alternative pathway more effectively than less Fc-reactive strains (e.g. types 7, 4, 12, 9, 13, and 23)*, but classical complement pathway activation is independent of such Fc-reactivity (Stephens et al. 1977, * American nomenclature?).

The membrane attack complex is formed normally and is inserted into the murein layer, but this layer is too thick in pneumococci to cause lysis (Joiner et al. 1983).

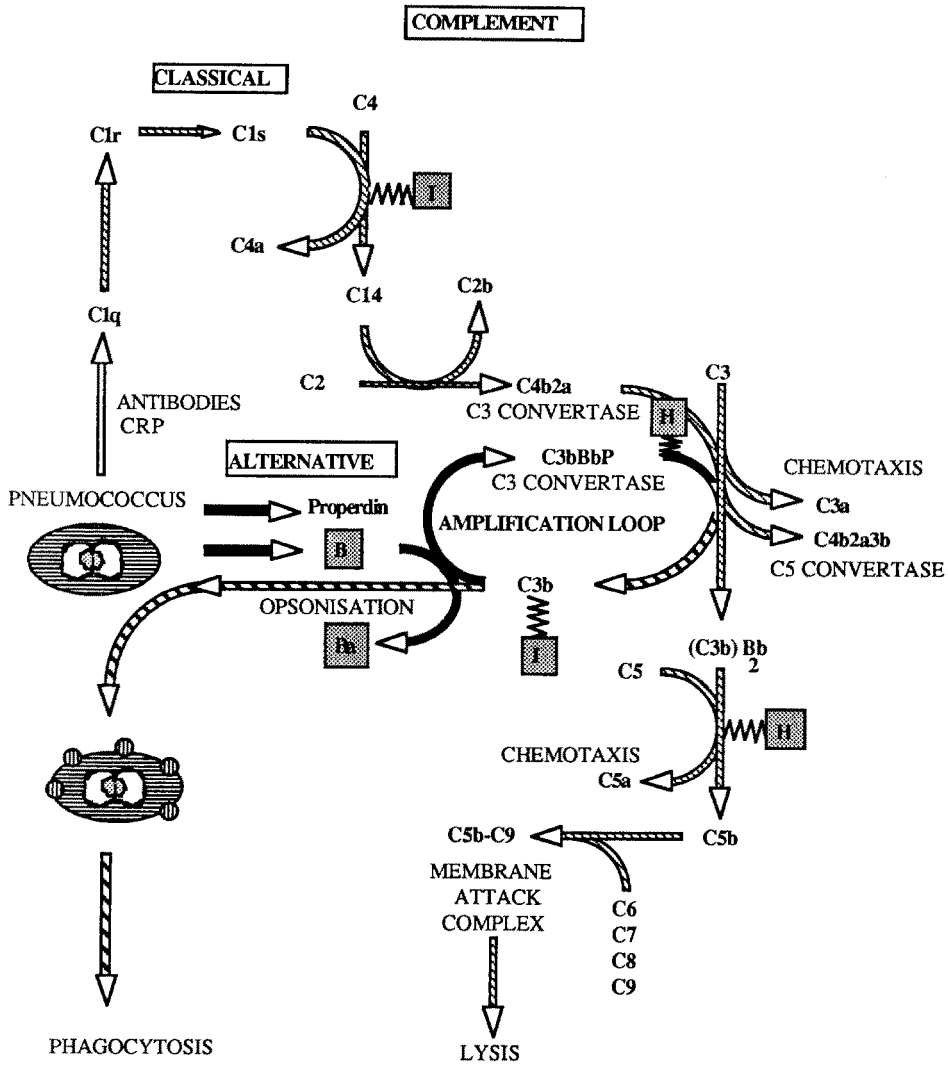
3.1. Classical complement pathway

Specific antibodies of the IgG class can indirectly stimulate opsonization through a conformational change in the Fc portion of the antibody, which activates the first component of complement C1q. Cell wall components show an antibody-independent C1q-binding capacity with teichoic acid being the most efficient constituent in this respect (Prellner 1981), but require antibodies for complement activation. Subsequent, the classical complement pathway is activated, resulting in deposition of the opsonin C3b on the surface of the pneumococcus (Scheme 2). C3b deposited on to encapsulated pneumococci mediated by anti-capsular antibodies is a more efficient opsonin than C3b deposited by anti-cell-wall antibodies, which interacts poorly with C3b receptors (Brown et al. 1983b). The increased virulence of encapsulated versus unencapsulated pneumococci in the presence of anti-cell wall antibodies may be explained by the interference of the capsule with the recognition of cell-wall-bound C3b molecules by phagocyte receptors. C3b binds to the cell wall of unencapsulated pneumococci in an amide linkage while in many encapsulated types an ester linkage is formed. Amide-linked C3b is a more potent stimulus for phagocytic activity than ester linkage, which explains the rapid phagocytosis of unencapsulated pneumococci (Hostetter 1986).

After covalent binding of C3b, factor I cleaves C3b in the presence of factor H to iC3b. The *S. pneumoniae* types 3, 4, 6A, and 14 differ in the amounts and sites of covalently bound C3b, and in degradative processing to iC3b and C3d. Complement proteins C3b and C3d bind to the surfaces of types 3 and 4, while iC3b is found on the surfaces of all four capsular types (Hostetter 1986). PMN's have membrane receptors for C3 fragments C3b, iC3b and C3d. Complement receptor type 1 (CR1), the C3b receptor, is a single-chain glycoprotein (MW 205–260 kD), expressed on erythrocytes, neutrophils, monocytes, B-lymphocytes, glomerular podocytes, follicular dendritic cells and some T-lymphocytes. CR1 binds preferentially to C3b, but it can also bind to iC3b and C4b. Complement receptor type 3 CR3 binds only iC3b and is expressed on neutrophils, monocytes, large granular lymphocytes and follicular dendritic cells.

CR3 is the major phagocytic receptor for virulent pneumococci in the non-immune host (Gordon et al. 1986). In the early stages of host defense against infection, before the synthesis of type-specific anti-capsular antibodies, iC3b interactions with CR3 play a central role. Types 6 and 14, which bear iC3b exclusively bound to the capsule, are phagocytized primarily through the CR3 receptor, even in the absence of anti-capsular antibodies.

Pneumolysin activates the human classical com-



Scheme 2. Diagrammatic representation of the mechanisms by which complement can be activated by microorganisms (no role for the membrane attack complex C5b-C9 has been reported for the elimination of pneumococci).

plement pathway, and reduces serum opsonic activity for *S. pneumoniae* by inhibiting the antimicrobial activities of PMN's (Paton et al. 1984).

3.2. Alternative complement pathway

The natural host defense to *S. pneumoniae*, i.e. in the absence of specific antibodies, is mediated by the complement opsonin C3b activated through the alternative complement pathway. C3b-coated pneumococci are efficiently removed from the circulation (Winkelstein 1984), and only 10–30% of pneumococcal pneumonias are associated with bacteremia (Schwartz 1982). Many types (e.g. types 7F, 12F, 14, 18F, 23F, and 25F) readily activate the alternative pathway (Giebink et al. 1977; Fine 1975). The alternative complement pathway may be activated in the absence of antibodies by bacterial cell wall substances like lipopolysaccharide (LPS, not present in pneumococci), peptidoglycan, teichoic acid, or lipoteichoic acid (Hummell et al. 1985). C3-convertase activity is generated directly, leading to C3b, which binds to the microbe, and C3a, a chemotactic agent that attracts polymorphs. Other types (e.g. types 1, 3, 4 and 8)

lack the intrinsic ability to activate the alternative pathway although types 3, 4 and 8 can do so in concert with specific antibodies (Fine 1975). Polysaccharides may either decrease or increase the binding of factors H and I for C3b, resulting either in an increased formation of the alternative pathway convertase or a further breakdown of C3b, respectively (Brown et al. 1983c). Breakdown may result in iC3b formation, which is opsonically active, or in opsonically inactive fragments. A factor which contributes to the varying abilities of pneumococcal types to activate the alternative pathway may be the different mechanical barrier imposed by the particular configuration of the capsular material, which hinders exposure of the teichoic acid binding sites for C3b. Properdin and the alternative complement pathway play an important role in efficient granulocyte phagocytosis of *S. pneumoniae* types 6A, 14, 19F, 23F and 35, in the most pronounced way in types 14 and 23F (Braconier et al. 1983a).

4. C-Reactive protein, CRP

An acute phase reactant is C-reactive protein (CRP), produced in large amounts by the liver to inflammatory stimuli before the primary antibody response). The activity of CRP requires a functioning complement system and may postpone the development of fatal levels of pneumococci in the blood (Horowitz et al. 1987). CRP is multivalently binding with several ligands, and is reactive with phosphorylcholine, present in the C-polysaccharide from the pneumococcal cell wall (Volanakis & Kaplan 1971; Mold et al. 1981). CRP-pneumococcal C-polysaccharide complexes are solubilized by complement protein C4 (Volanakis & Narkates 1983). Binding of CRP to types 3, 6 and the unencapsulated strain R36a leads to activation of the classical complement pathway, but inhibits alternative pathway activation (increases factor H binding to C3b) (Mold et al. 1982, 1984). The opsonophagocytic response of PMN's is not influenced by type 6 or R36a, but is inhibited by type 3 pneumococci, although injection of mice with CRP has a protective effect against infection with *S. pneumoniae* types 3 and 4, accompanied by increased splenic sequestration of the bacteria (Mold et al. 1981, 1982, 1984). However, even complement-depleted or splenectomized mice are protected, indicating that the protective effect of CRP is independent from both the complement system and clearance from the spleen (Nakayama et al. 1983). CRP does not increase opsonic activity for types 4 or 7F, and might promote complement-independent phagocytosis (Chudwin et al. 1985a). It is not unlikely, that in splenectomized animals the protective effect of CRP is mediated by an enhanced clearance by the liver.

Enhancement of PMN opsonization of *S. pneumoniae* type 27, which has a phosphorylcholine residue in its capsular repeating oligosaccharide unit (Table 2; 41, Bennett & Bishop 1977), is dependent on CRP and the classical complement pathway (Holzer et al. 1984). CRP and complement opsonin C3b bind to *S. pneumoniae* type 4 (and probably also to other virulent types) only in the cell wall region, but binding of CRP to type 27 occurs at both capsule and cell wall. Anti-S4 antibodies deposit C3b in the capsule while anti-S27 and CRP-S27 complexes deposit C3b in both capsule and the cell wall. The role of phosphorylcholine as a virulence diminishing factor should be investigated in more detail. It may explain the low virulence of unencapsulated pneumococci, type 27 and probably also type 24A and groups 28 and 32 pneumococci (Sørensen et al. 1984).

In patients with severe systemic disease, the incidence of alternative pathway deficiencies is relatively high and associated with high CRP levels (Rabinovich et al. 1986).

III. Immune response to polyvalent pneumococcal capsular polysaccharide vaccine

The antibody response of normal subjects to polyvalent pneumococcal polysaccharide vaccine shows a long-term persistence of vaccine-induced type specific pneumococcal antibodies (Mufson et al. 1983, 1987). Only mild adverse reactions such as local pain and erythema have been observed upon immunization. Patients receiving the vaccine sub-

Table 2. Repeating structures of *Streptococcus pneumoniae* Capsular Polysaccharides.

Serotype			
C	→6)-β-D-Glc*p*-(1→3)-α-Sug*p*-(1→4)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*N-(1→1)-D-Rib-ol-(5-PO_4^-→[a] 6 PO_4^--$CH_2CH_2N^+(CH_3)_3$	(1)	(Jennings et al. 1980)
S1	→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→[a] (+0.3 OAc)	(2)	(Lindberg et al. 1980)
S2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→ 2 1 α-D-Glc*p*A-(1→6)-α-D-Glc*p*	(3)	(Jansson et al. 1988)
S3	→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→	(4)	(Reeves & Goebel 1941)
S4	→3)-β-D-Man*p*NAc-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-(1→ 2 3 X H_3C COOH	(5)	(Jones & Currie 1988)
S5	→4)-β-D-Glc*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-Sug*p*-(1→[b] 3 1 α-L-Pne*p*NAc-(1→3)-β-D-Glc*p*A	(6)	(Jansson et al. 1985)
S6[A]	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Rib-ol-(5-PO_4^-→	(7)	(Rebers & Heidelberger 1961)
S6[B]	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5-PO_4^-→	(8)	(Kenne et al. 1979)
S7[F]	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 2 2 4 1 OAc 1 β-D-Gal*p* α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*	(9)	(Moreau et al. 1988)
S7[A]	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 2 4 OAc 1 α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*	(10)	(Jansson et al. 1988d)
S7[B]	--		
S7[C]	--		
S8	→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→	(11)	(Jones & Perry 1957)
S9[A]	→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→	(12)	(Bennett & Bishop 1980)
S9[L]	→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*NAc-(1→	(13)	(Richards et al. 1984)
S9[N]	→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*NAc-(1→	(14)	(Jones et al. 1985)
S9[V]	→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→ +OAc +OAc	(15)	(Perry et al. 1981)
S10[F]	→6)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5-PO_4^-→ 6 1 β-D-Gal*f*	(16)	(Perry et al. 1980)
S10[A]	→6)- -D-Gal*f*-(1→3)- -D-Gal*p*-(1→4)- -D-Gal*p*NAc-(1→3)- -D-Gal*p*-(1→2)-D-Rib-ol-(5-PO_4^-→ 6 (+OAc) 1 β-D-Gal*f*	(17)	(Rao et al. 1966)
S11[F]	→6)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 3 4 2 (+0.5 OAc) $(AcO)_{0.5}$ PO_4^--1-Rib-ol OAc	(18)	(Richards et al. 1985)
S11[A]	→6)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→ 2/3 4 (+OAc) AcO PO_4^--1-Glyc-ol	(19)	(Kennedy et al. 1969)
S11[B]	→6)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 3 4 AcO PO_4^--1-Rib-ol	(20)	(Richards et al. 1985)
S11[C]	→6)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 3 4 AcO PO_4^--1-Glyc-ol	(21)	(Richards et al. 1985)

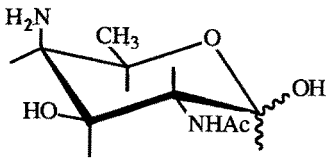
Table 2. Continued.

Serotype		
S12^F	→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→ 3 3 1 1 α-D-Gal*p* α-D-Glc*p*-(1→2)-β-D-Glc*p*	(22) (Leontein et al. 1981)
S12^A	→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→ 3 3 1 1 α-D-Gal*p*NAc α-D-Glc*p*-(1→2)-β-D-Glc*p*	(23) (Leontein et al. 1983)
S13	→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*f*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Rib-ol-(1-PO_4^-→ 2/3 OAc	(24) (Watson et al. 1972)
S14	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 1 β-D-Gal*p*	(25) (Lindberg et al. 1977)
S15^F	β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 (+ 2 OAc) 1 →3)-α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4^--$CH_2CH_2N^+(CH_3)_3$	(26) (Perry et al. 1982)
S15^A	β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 1 →3)-α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4^--2-Glyc-ol	(27) (Carroff & Perry 1984)
S15^B	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 1 +0.7 OAc α-D-Gal*p*-(1→2)-β-D-Gal*p* -3-PO_4^--R 80% R=H 20% R=$CH_2CH_2N^+(CH_3)_3$	(28) (Jansson et al. 1987)
S15^C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 1 α-D-Gal*p*-(1→2)-β-D-Gal*p* -3-PO_4^--R 80% R=H 20% R=$CH_2CH_2N^+(CH_3)_3$	(29) (Jansson et al. 1987)
S16^F S16^A	contains Glc, Gal, Rha, GlcNAc, GalNAc, and Glyc-ol-PO_4^-	(Shabarova et al. 1962)
S17$^{b'}$	→4)-α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→2)-D-Ara-ol-PO_4^-→ 4 1 α-D-Gal*p*	(30) (Perry et al. 1983)
S17^A	→3)-β-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→ AcO2 4 2 1 1 β-D-Gal*p* α-D-Glc*p*	(31) (Jansson et al. 1983)
S18^F	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→ 2 3 2 1 PO_4^- -1-Glyc-ol OAc AcO6-α-D-Glc*p*	(32) (Jansson et al. 1988a)
S18^A	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-GlcNAc*p*-(1→3)-β-L-Rha*p*-(1→ 2 3 1 PO_4^- -1-Glyc-ol α-D-Glc*p*	(33) (Jansson et al. 1988b)
S18^B	--	
S18^C	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→ 2 3 1 PO_4^- -1-Glyc-ol AcO3-α-D-Glc*p*	(34) (Lugowski & Jennings 1984)
S19^F	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1-PO_4^- →	(35) (Lee & Fraser 1980; Ohno et al. 1980)
S19^A	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1-PO_4^- →	(36) (Katzenellenbogen & Jennings 1983; Lee et al. 1987)
	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1-PO_4^- → 2 3 β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→PO_4^- PO_4^- -1)-α-L-Fuc*p*	(37) (Lee & Fraser 1980; Lee et al. 1987)
S20	→6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→3)-α-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Glc*p*NAc-(1-PO_4^- → +OAc 4 1 β-D-Gal*f*	(38) (Richards et al. 1983)

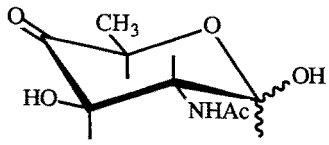
Table 2. Continued.

Serotype			
S21	contains Glc, Gal, and GlcNAc		(Shabarova et al. 1962)
$S22^F$	→4)-β-D-Glc*p*A-(1→4)-β-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→3)-α-D-Gal*f*-(1→2)-α-L-Rha*p*-(1→ $(AcO)_{0.8}$ 2 3 1 α-D-Glc*p*	(39)	(Richards & Perry 1986)
$S22^A$	--		
$S23^F$	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→ 2 3 1 PO_4^- 2-Glyc-ol α-L-Rha*p*	(40)	(Richards & Perry 1988)
$S23^A$	--		
$S23^B$	--		
$S24^F$	contains Glc, GlcNAc, Rha, Rib, and Rib-ol-PO_4^-		(Shabarova et al. 1962)
$S24^A$	contains phosphorylcholine		(Sørensen et al. 1984)
$S24^B$	--		
$S25^F$	contains Gal, GalA, GalNAc, and GlcNAc		(Das et al. 1976)
$S25^A$	--		
S27	→3)-β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 4 6 2 X PO_4^--$CH_2CH_2N^+(CH_3)_3$ H_3C COOH	(41)	(Bennett & Bishop 1977)
$S28^F$	contains phosphorylcholine, Glc, Rha, and Glyc-ol-PO_4^-		(Sørensen et al. 1984; Shabarova et al. 1962)
$S28^A$	contains phosphorylcholine		(Sørensen et al. 1984)
S29	→4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Gal*f*-(1→1)-Rib-ol-(5-PO_4^-→	(42)	(Kenne & Lindberg 1988)
S31	→2)-β-L-Rha*p*-(1→3)-β-D-Gal*f*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→	(43)	(Batavyal & Roy 1983)
$S32^F$	contains phosphorylcholine, Glc,Gal, and Rha		(Sørensen et al. 1984; Shabarova et al. 1962)
$S32^A$	contains phosphorylcholine		(Pazur et al. 1983) (Sørensen et al. 1984)
$S33^F$	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*-(1→ 2 2 1 $(OAc)_{0.4}$ α-D-Gal*p*	(44)	(Richards et al. 1984)
$S33^A$	--		
$S33^B$	→)-β-D-Glc*p*-(1→5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5-PO_4^-→ 2 1 α-D-Gal*p*	(45)	(M.J. Watson 1974)
$S33^C$	--		
S34	→3)-β-D-Gal*f*-(1→3)-α-D-Glc*p*-(1→2)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5-PO_4^-→ 6 OAc	(46)	(Chittenden et al. 1968)
$S35^F$	--		
$S35^A$	--		
$S35^B$	--		
$S35^C$	--		
S36	--		
S37	→3)-β-D-Glc*p*-(1→ 2 1 β-D-Glc*p*	(47)	(Adeyeye et al. 1988)
S38	--		
S39	--		
S40	--		
$S41^F$	--		
$S41^A$	--		
S42	--		
S43	--		
S44	--		
S45	→3)-α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→2)-α-L-Rha*p*-(1→ 6 4 1 1 α-D-Gal*p* β-D-Glc*p*NAc-(6-PO_4^--1-Glyc-ol	(48)	(Moreau et al. 1988)
S46	contains Gal, GalNAc, GlcNAc, and FucNAc		(Benzing et al. 1981)
$S47^F$	--		
$S47^A$	--		
S48	--		

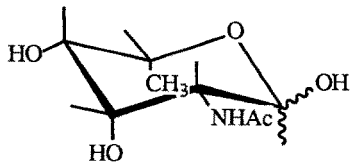
Table 2. Continued.



[a] D-Sug = 2-acetamido-4-amino-2,4,6, trideoxy-D-Galactose



[b] D-Sug = 2-acetamido-2,6- dideoxy-D-Xylo-hexos-4-ulose



L-PneNAc = 2-acetamido-2,6-dideoxy-L-Talose

cutaneously have fewer side effects (Pönkä & Leinonen 1982). Decreased immune responses, observed in adults immunized with 14 valent Pneumovax©, has been ascribed to contaminant blood-group-A-like substance (Nurmi & Koskela 1988). The 23 valent vaccine contains no blood-group-like substances, by better adaptation of the growth medium. A second dose after a one or two year interval does not further increase antibody levels (Mufson et al. 1984). The interval between repeated doses should be at least five years, but revaccination after 10 years gives a lower rise in anticapsular antibodies than primary immunization, and revaccination was not recommended (Centers for Disease Control 1984). More recent data suggest revaccination should be considered for persons who received the 14-valent vaccine if they are at the highest risk of fatal pneumococcal infection, for persons who show to have a rapid decline in pneumococcal antibody levels, and for children with nephrotic syndrome, asplenia, or sickle cell anaemia (Centers for Disease Control 1989).

Patients with bacteremia are probably the most likely candidates to benefit from previous stimulation of circulating antibodies by a vaccine. As many as 10–30% of patients with pneumococcal pneumonia also have bacteremia (Schwartz 1984). Elevated prevaccination levels may reduce the potential benefit of vaccination (Williams & Mosher 1986).

1. Pneumococcal vaccine in infancy and childhood

Invasive diseases caused by encapsulated bacteria occur with the highest frequency in infants and children after the decline of maternally derived IgG antibody levels, i.e. after 6 months. The pneumococcal acquisition rate of infants shows a strong seasonal influence, with a marked winter peak, while fluctuations in the carriage rate are less pronounced (Gray et al. 1982). Rates of pneumococcal carriage may range from 80–100% and involve more than a single type (Austrian 1989). The acquisition rate increases with age, while the duration of carriage decreases.

In infants under two, many of the pneumococcal polysaccharides are not highly immunogenic, including the 4 most common types in otitis media (e.g. types 6AB, 14, 19F and 23F) (Mäkelä et al. 1983). Since the highest incidence rates of otitis media occur in early childhood, it is important to improve the immunogenicity of pneumococcal vaccine for this age group. Recurrences of otitis media after pneumococcal vaccination were significantly rarer in children over 9 months old (with the exception of group 6 infections), while younger children were not protected (Karma et al. 1982). No difference in response to vaccination has been observed in otitis media-prone children compared with a control group (Kalm et al. 1986; Prellner et al. 1984), except for significantly lower antibody levels against type 6A and 19F (Kalm et al. 1984). The increase in serum antibody levels in children over 6 months old to most of the polysaccharides is satisfactory, except for group 6 (Mäkelä et al. 1980; Prellner et al. 1984). Moreover, complement com-

ponent C1q concentrations were relatively low in the acute phase of otitis media. The pneumococcal vaccine has been reported to be effective in reducing child morbidity and mortality from acute lower-respiratory tract infections, although responses vary markedly with age at the time of immunization, and the serotype tested (Riley et al. 1986). Infants frequently made low levels of type-specific antibody in response to colonization and infection, but otitis media occurred even in the presence of specific antibodies (Gray & Dillon 1988). An IgG antibody response to type 3 occurs as early as 2 months; little or no response was noted in either IgG or IgM with types 6A, 18C, and 19F; an intermediate IgM antibody response was observed for type 23F (Barrett et al. 1984). Multiple or 'booster' immunizations of infants with these immunogens do not elicit sufficient antibody formation for protection against pneumococcal diseases and the antibody levels decrease rapidly after vaccination (Koskela et al. 1986). Protective efficacy is better in children over 2 years old, when IgG2 antibody levels are increasing (Schatz & Barrett 1987). No difference was found in nasopharyngeal carriership in children (6 months – $4^1/_2$ years) between vaccinees and controls. Elevated homotypic serum antibody concentrations are present in children who carried types 18C, 19F, and 23F, but not for types 6A and 14 (Douglas et al. 1986). Group 6 pneumococci were carried more frequently and for longer periods than groups 19 and 23 (Rosén et al. 1985).

An important host defense component for newborns is provided by the maternal-fœtal transfer of IgG antibodies. Lower levels of type 7F specific IgG antibodies were found in cord serum than in maternal blood samples, and no IgM (Chudwin et al. 1985b). Pneumovax© in pregnant women gives no fœtal immunization, since no IgM is found in umbilical cord blood (Vincent-Ballereaux et al. 1985). Women who gave birth to infants with neonatal group B *Streptococcus* septicemia showed significantly higher preimmune IgM levels against 3 of 6 pneumococcal antigens, and lower IgG levels against S14 (Christensen et al. 1985a). These mothers might differ in their capacity to switch from IgM to IgG production.

In children under 2 years old at vaccination, cases of acute tonsillitis are reported more frequently than in older children (Christensen et al. 1985b).

The levels of type-specific antibodies increase with age after the second year to 8–10 years for most types including types 6A, 18C, and 19F, but the levels against types 14 and 23F continue to augment with ages 13–15 years (Windebank et al. 1987; Paton et al. 1986).

2. Pneumococcal vaccine in the elderly

Even with penicillin therapy, mortality from bacteremic pneumococcal pneumonia remains high (up to 55–60%) in elderly patients. Vaccination of the elderly with Pneumovax© to boost anti-capsular antibody levels, should therefore be considered as a means of preventing serious pneumococcal disease, particularly in those with chronic debilitating disease and those who are institutionalized (Schwartz 1982). The clinical effectiveness of Pneumovax© in the immunocompetent elderly persons has been estimated to be 70% (Sims et al. 1988), and the vaccine should be given when the patient is not acutely ill. Vascular disease affecting the heart, peripheral, and/or cerebral vessels is a major underlying condition among the oldest patients (Burman et al. 1985).

Elderly persons, especially those of 80 years and older, have lower prevaccination anti-capsular antibody levels than young healthy controls (Roghman et al. 1987), except for type 3, but a severalfold increase has been observed after vaccination (Leinonen 1982). A remarkably wide variation in antibody levels against different serotypes in pre- and postvaccination sera exists (prevacc. S9N lowest, S1 highest counts; postvacc. S6A lowest, S7F highest). The IgM isotype responses of elderly subjects to the Pneumovax© are poor and significantly lower for 6 of the 14 types tested (e.g. types 1, 4, 7F, 12F, 14 and 25F) (Ruben & Uhrin 1985). Elderly women have lower prevaccination levels of anti-capsular antibody, but respond better to vaccination than men. They also lose their acquired antibodies faster (Roghman et al. 1987).

3. Pneumococcal vaccine in high-risk groups

The risk of mortality and morbidity due to *S. pneumoniae* infections increases among patients with chronic debilitating conditions like alcoholism, liver cirrhosis, chronic heart disease, renal failure, broncho-pulmonary disease, malignancy, diabetes mellitus, and those convalescing from severe diseases, or patients with functional or anatomic asplenia (e.g. sickle cell disease) (Burman et al. 1985). These patients may, therefore, benefit from vaccination. However, the response to Pneumovax© in these patients is variable, and in some cases even disappointing, i.e. the vaccine fails to provide protection. The results of vaccination of patients with the various disorders mentioned above, particularly as regards to protection, are summarized in the next paragraphs.

3.1. Bronchopulmonary disease

It has been well established that patients with chronic bronchopulmonary disease, i.e. bronchial asthma, chronic bronchitis, emphysema, bronchiectasis, active tuberculosis, primary or secondary pulmonary tumours, or post-operative, post-tuberculous, or traumatic reduction of lung parenchyma, run an increased risk of severe pneumococcal infection. Pneumococcal pneumonia complicated by empyema has been associated with a high mortality (Gransden & Eykyn 1988).

Three years after vaccination, only slightly higher IgG antibody titres have been found in patients with chronic obstructive lung diseases than in unvaccinated patients, which indicates that no long-term protection is obtained (Kraus et al. 1987). The benefit of vaccination in patients with chronic lung disease is possibly limited (Leech et al. 1984), and not recommended for all patients (Williams & Mosher 1986). In bronchitics, the opsonizing activity for 6 of 9 serotypes was even lower after vaccination (Musher et al. 1986), emphasizing the need for alternative strategies to maximize the protective effect of the pneumococcal vaccine, in the population at greatest risk.

3.2. Hepatic disease

Mortality due to pneumococcal pneumonia is especially high in the presence of leukopenia (Austrian & Gold 1964) associated with alcoholism. Chronic active hepatitis and primary biliary cirrhosis also predispose to pneumococcal infections, although they are not associated with alcoholism. The antibody response and opsonic titres of cirrhotic patients after inoculation shows an adequate response to the pneumococcal vaccine (Pirovino et al. 1984), but its clinical efficacy remains to be proven (Simberkoff et al. 1983).

3.3. Renal failure

The degree of renal function impairment affects antibody levels following pneumococcal immunization (Rytel et al. 1986). The primary antibody response of adult hemodialysis patients has been reported to be delayed, and to be significantly lower $3^1/_2$ years after immunization, than in controls (Simberkoff et al. 1980). Two years after Pneumovax© was given, the lowest antibody levels were to types 4, 6A, and 19F (Linnemann et al. 1986). Revaccination gave a lower increase in antibody levels than primary vaccination. Renal allograft recipients have stronger antibody responses (especially of the IgM class) than azotemic and hemodialysis patients (Nikoskelainen et al. 1985). The decline in antibody titres is not significantly different from those of control subjects, and the effect of azathioprine and corticosteroid medication on antibody levels is small (Rytel et al. 1986). In kidney transplant recipients, the increase in antibody levels for type 3 and 6A antigens has little effect on opsonic activity (Bortolussi et al. 1981).

In children with nephrotic syndrome, who have a defective bacterial opsonization associated with decreased serum concentrations of the alternative complement pathway factor B, Pneumovax© elicits a serological response, but fails to protect (Moore et al. 1980).

3.4. Autoimmune diseases

In patients with systemic lupus erythematosus, post-immunization antibody titres were significantly lower than in healthy controls (McDonald et al. 1985). Two years after vaccination the responses to S4, S7F, and S18C are reduced, while after three years a significantly lower response to S1, S7F,

S9N, and S18C has been observed. A case of vaccine failure due to infection with *S. pneumoniae* type 14 has been reported.

Antibodies to pneumococcal capsular polysaccharides as well as IgG2 and IgG4 were undetectable in a patient with Sjögren's syndrome and recurrent bacteremic pneumococcal infection. A prompt antibody response to some types (1, 9N, and 14) was induced by vaccination, even in the IgG2 subclass (Matter et al. 1985). Suppression of the B-cell response to pneumococcal antigens presented through mucous membranes or during tissue invasion may be circumvented by subcutaneous vaccination.

3.5. Immune deficiency

Patients with congenital or acquired deficiencies of the complement system are predisposed to more frequent and severe pneumococcal infections.

Combined IgA and IgG2 deficiency has been associated with an increased susceptibility to infection, and often an impaired lung function (Lane & MacLennan 1986). Analysis of the complement profile in an adult patient with IgG2, IgG4 and IgA deficiency (Prellner et al. 1985) showed no impairment of either the classical or alternative complement pathway. Together with a poor IgM and IgG response, decreased levels of C1q with an excess of C1r-C1s complexes were observed, while C4 was low.

Patients with isolated IgA deficiency show a normal rise of antibody concentrations of all IgG subclasses in response to S14, and a rise of both IgG2 and IgG4 was observed to S1. In healthy controls only IgG4 antibody concentrations rise significantly. Moreover IgA-deficient individuals show enhancement of IgG1 and IgG3 antibody responses, which suggests altered regulation of the subclass and isotype switching processes (Roberton et al. 1989).

Anti-polysaccharide responses in surgical patients are normal, but have no relationship to the outcome of a septic process (Nohr et al. 1986).

Out of 35 AIDS patients, five had pneumococcal infections (2 infections with type 4, 1 with type 22, 1 with type 17, and 1 of unknown type) (Simberkoff et al. 1984). To type 4, no opsonic titres were detected, despite prior vaccination. Responses to pneumococcal vaccine were impaired in all antibody classes in patients with AIDS and in asymptomatic seropositive men (although the mean-fold increases are similar to control groups) (Janoff et al. 1988). Significant lower responses were found to S9N and S18C (Huang et al. 1987).

3.6. Splenectomized patients

Splenectomized patients and those with functional asplenia, such as in sickle cell anaemia, show increased susceptibility to bacterial infection. The main underlying factors involve decreased clearance of bacteria from the blood due to an impaired phagocytic capacity and/or inability to mount a sufficiently rapid antibody response (Hammarström & Smith 1986). After splenectomy, there are also reduced levels of properdin, leukophilic gammaglobulin and the phagocytosis stimulating tetrapeptide tuftsin (Hosea 1983).

Decreased clearance of bacteria like type 3 pneumococci, which are cleared primarily by the liver, has been attributed to impairment of hepatic clearance and/or inability of the liver to compensate for the loss of the splenic function (Grover & Loegering 1984). Splenectomized patients are markedly deficient in their capacity to remove IgG-coated particles from the bloodstream, while C3b-coated particles are cleared normally, but are released back into the bloodstream as a result of inactivation of C3b (Hosea et al. 1981a). There is still controversy over the issue whether C3b only mediates binding to phagocytes but not ingestion. In sickle cell disease, the serum opsonic activity for *S. pneumoniae* mediated by both alternative and classical pathways is depressed (Bjornson et al. 1985), and an antibody defect may contribute to the reduction in opsonic function (Bjornson & Lobel 1987). Both purified IgG and $F(ab')_2$ fragments of the IgG preparation restore alternative pathway-mediated opsonization of *S. pneumoniae*, in serum of patients with sickle cell disease (Bjornson & Lobel 1986).

Splenectomy causes a persistent immune deficiency of circulating B-cells capable of producing

anti-polysaccharide IgM (DiPadova et al. 1985). The rise of type-specific IgG and IgM levels is much slower, while absolute levels are also lower than in controls (Hosea et al. 1981b). The response in splenectomized children is significantly lower (Pedersen 1983) for types 2, 3, 8, and 12F. A significant antibody decline has been observed (especially for types 1, 4, 6A, 7F, 8, 19F, and 23F) in children splenectomized because of trauma (Giebink et al. 1984). An important role for the spleen in the production of specific anti-carbohydrate antibodies (mostly IgG_2) has been suggested.

Pneumococcal vaccine is effective in improving the antipneumococcal defenses in splenectomized mice (Hebert 1989) or rats (Iinuma & Okinaga 1989), but not to the same degree as animals retaining their spleens. In splenectomized mice, antibody responses to S3 and S14 differ in dependence upon the spleen. Splenectomy resulted in a loss of early antibody responses to low or optimal doses of S3, but obliterated antibody responses to all doses of S14 (Cohn & Schiffman 1987). The dependence of S14 on the spleen is possibly due to the tendency of the antigen to become localized in the marginal zone in the spleen, while S3 is localized in the red pulp.

The pneumococcal vaccine is poorly immunogenic in splenectomized patients with Hodgkin's disease, who are heavily treated by chemotherapy and radiotherapy (Levine et al. 1979; Minor et al. 1979; Weitzman et al. 1978; Sullivan et al. 1978). Untreated patients however, respond like normal controls to vaccination (Levine et al. 1979). Better responses have been reported in such patients when vaccination had been performed prior to splenectomy (Donaldson et al. 1981; Gonzaga 1984). Timing of immunization in children before or after splenectomy has no impact on the antibody response, as long as the children are untreated, but is impaired if chemotherapy begins less than 10 days after immunization (Siber et al. 1986). Decline in antibody levels is significantly greater in patients with Hodgkin's disease, while booster immunizations failed to elicit any increase.

Despite good antibody responses after primary and booster immunizations (Weintrub et al. 1984) in children with sickle cell disease and penicillin prophylaxis, pneumococcal infections have been caused by types present in the vaccine (e.g. types 3, 6AB, (9A), 19F, and 23F) (John et al. 1984; Buchanan & Smith 1986).

3.7. Multiple myeloma

Impaired responses have also been found in patients with multiple myeloma (Lazarus et al. 1980), but less so among patients not receiving chemotherapy (Schmid et al. 1979). A rapid return to prevaccination antibody levels for 7 out of 14 antigens (e.g. S1, S3, S4, S6A, S8, S12F, and S19F) has been observed (Birgens et al. 1983). Patients with chronic myelogenous leukemia, not receiving chemotherapy, who were vaccinated prior to splenectomy, respond normally, whereas the response is significantly poorer in patients receiving chemotherapy (Donaldson et al. 1981; Brown et al. 1982a). The response improves with time after splenectomy (Landesman & Schiffman 1981). Multiple myeloma patients demonstrate a defect in C3b binding to S3 and decreased S14 and S25 binding (Cheson et al. 1984).

Lower pre- and post-immunization antibody titres, as compared with controls, have been found in patients with plasma cell dyscrasias (Shildt et al. 1981), while occasional rises in antibody titres occur.

Allogeneic bone marrow transplant recipients have lower type-specific IgG and normal IgM antibody levels. Immunization of donors does not provide effective prophylaxis (Giebink et al. 1986) to the recipient, and because vaccination of the recipient does not afford protection until at least 2 years after transplantation an alternative means of pneumococcal prophylaxis is highly desirable.

3.8. Diabetes

Patients with insulin-dependent diabetes respond adequately to pneumococcal vaccination (Beam et al. 1980), and may be not at a significantly increased risk of developing pneumococcal pneumonia (Schwartz 1982). However, the presence of diabetes as coexisting illness increases the risk of mortality (Mufson et al. 1974).

IV. Immunochemistry of *Streptococcus pneumoniae* cell wall components

The pneumococcal cell wall is active in inducing inflammation during pneumococcal meningitis (Tuomanen et al. 1985), and may contribute to chronic otitis media (Ripley-Petzholdt et al. 1988). Both major cell wall components present in all pneumococci, teichoic acid and peptidoglycan, contribute to this activity (Tomasz 1981). Teichoic acid has the highest specific activity of all the cell wall fractions, with peak activity 5 hrs after immunization, compared with 24 hrs for peptidoglycan containing fractions. Teichoic acid is also the most important stimulus for secretion of interleukin-1 (IL-1) from monocytes, but not of other mediators of inflammation like tumor necrosis factor (TNF) (Riesenfeld-Orn et al. 1989). Both cell wall components, peptidoglycan and teichoic acid, have the capacity to bind complement component C3b (Hummel et al. 1981).

Teichoic acid/C-polysaccharide

The ribitol-phosphate teichoic acid or C-polysaccharide is a linear polymer (Table 2; 1) of unusually complex structure, containing phosphorylcholine (Mosser & Tomasz 1970; Jennings et al. 1980a), through which the interaction with CRP occurs (Volanakis & Kaplan 1971), and which triggers the alternative complement pathway (Winkelstein & Tomasz 1978). C-polysaccharide is uniformly distributed on both the inside and outside of the cell walls, which thickness is varying with the strain (Sørensen et al. 1988). Phosphorylcholine on the cell wall acts as both polyclonal activator and specific antigen, each by its own mechanism (Bach et al. 1984). Moreover, phosphorylcholine residues also play a basic role as receptors for pneumococcal bacteriophage Dp-1 (López et al. 1982).

Anti-phosphorylcholine antibodies occur naturally in human serum, as infants generally develop antibodies to phosphorylcholine in response to pneumococcal carriage and infection (Gray et al. 1983). Antibodies to C-polysaccharide give no protection against pneumococcal infections, as the capsule prevents binding of the anti-C-polysaccharide antibodies. These antibodies may be important for the elimination of decaying pneumococci. Some anti-phosphorylcholine antibodies protect mice against challenge with certain, but not all, pneumococcal types (Szu et al. 1983; Briles et al. 1981a; Yother et al. 1982). IgM and IgG, but not IgA anti-phosphorylcholine monoclonal antibodies, protect mice against challenge with type 3 pneumococci (Briles et al. 1981b). Anti-S27 antibodies bind phosphorylcholine but are not protective against challenge with, for example, types 5, 6A and 6B organisms (Winkelstein & Tomasz 1978). The protective activity of phosphorylcholine-binding antibodies is related to their additional specificity for the sugar backbone of the C-polysaccharide or to binding of unidentified components of pneumococci and to their activation of complement (Szu et al. 1983). However, a protein conjugate of C-polysaccharide with BSA (prepared with the bifunctional agent SPDP) elicits antibodies to the C-polysaccharide in rabbits, but no phosphorylcholine-specific antibodies have been detected (Szu et al. 1986). Antibodies to the C-polysaccharide backbone fail to protect mice against challenge with type 3 or type 6A pneumococci, and no immunity against infection with encapsulated pneumococci is conferred. A monoclonal antibody that reacts with epitopes other than phosphorylcholine protects mice from infection with a type 3 strain, but not from type 5 or 6A strains (McDaniel et al. 1987).

Immunological heterogeneity of soluble cell wall-like polysaccharide and C-polysaccharide of different pneumococcal strains has been reported to reside in the mucopeptide portion of the molecule or in the region of its attachment to the teichoic acid moiety and not in the teichoic acid fraction (Schiffman et al. 1971).

Lipoteichoic acid/F-polysaccharide

The pneumococcal Forssman antigen or F-polysaccharide is the species-specific membrane-associ-

ated lipoteichoic acid antigen, which activates the alternative complement pathway but fails to induce immunity in mice against pneumococcal infections (Au & Eisenstein 1981b). Lipid is covalently attached to the phosphorylcholine-containing teichoic acid, which composition is similar, if not identical with the wall teichoic acid (Table 2; 1) (Fujiwara 1967). The F-polysaccharide is a powerful autolysin-inhibitor (Horne & Tomasz 1985; Briese & Hakenbeck 1985). The presence of choline is required for the inhibitory interaction with the pneumococcal autolysin (Giudicelli & Tomasz 1984), which is a *N*-acetyl-muramoyl-l-alanine amidase (murein hydrolase), essential for the cell separation process (Mosser & Tomasz 1970).

Peptidoglycan (murein)

Peptidoglycan or murein is a common component of bacterial cell walls, and is the target of penicillin and cephalosporin antibiotics. Peptidoglycan chains released from penicillin-treated pneumococci contain no teichnoic acid (Fischer & Tomasz 1985). Some of the symptoms of bacterial disease are induced by murein (Tuomanen 1986). The structural units are disaccharide tetrapeptides, interlinked by peptide bonds, which form complex cross-links (Garcia-Bustos et al. 1987). In addition to usual stem peptide components of pneumococcal cell walls (D and L-alanine, L-lysine, and D-isoglutaminic acid), significant amounts of serine, aspartate and glycine are present as inter-peptide bridges. Penicillin-resistant pneumococci show an altered peptidoglycan structure, containing more hydrophobic peptides carrying dialanyl or alanylserine cross-links (Garcia-Bustos et al. 1988). Muramyl dipeptide (MDP), the breakdown product of bacterial cell walls, is known as a powerful immunestimulator.

The purpura-producing principle from pneumococcal cell walls is a water soluble, high-molecular-mass (26 kD) peptidoglycan-teichoic acid complex, generated by the action of the pneumococcal autolysin on the bacterial cell wall (Chetty & Kreger 1985). Autolysin-negative mutants show reduced virulence for mice (Berry et al. 1989b), which indicates a role for autolysin in pathogenesis. Autolysin may act, besides by the release of highly inflammatory cell wall breakdown products, by lysing a proportion of the invading pneumococci, so toxins like pneumolysin and neuraminidase are released.

Protein antigens

Protein antigens are demonstrable on the surface of non-encapsulated pneumococci (Sørensen et al. 1988). Protein serotyping based on reactivity to six monoclonal antibodies subdivided pneumococci into 19 protein types, independent of capsular type, which may distinguish isolates with different virulence properties (Waltman et al. 1988). Surface protein A (PspA), which is present on at least 60% of pneumococci, is serologically variable in different strains of pneumococci (Briles et al. 1989). PspA has been shown to play a role in the virulence of pneumococci, and may elicit protective anti-pneumococcal antibodies (McDaniel et al. 1986, 1987). Antibodies to PspA have been shown to be effective at mediating blood and peritoneal clearance of pneumococci (Briles et al. 1989). PspB, a 64 kD surface protein is, like the R-antigen of group A and B streptococci, susceptible to pepsin and resistant to trypsin digestion, whereas PspA is susceptible to both (McDaniel & Briles 1988).

An extra layer of unknown nature is present in both rough and encapsulated strains (Sørensen et al. 1988).

V. Immunochemistry of *Streptococcus pneumoniae* capsular polysaccharides

The properties of polysaccharide antigens that determine their immunogenicity are poorly understood. Information about the contribution of charge, hydrophobicity, and conformational expression of epitopes within polysaccharides, would be very helpful in the development of new vaccines (Griffiss et al. 1987). In the next paragraphs a sum-

mary is given of the immunochemical properties of the capsular polysaccharides from the most important pneumococcal types.

1. Streptococcus pneumoniae types 3 and 8

S. pneumoniae capsular types 3 and 8 are two commonly isolated cross-reactive types, which are included in the pneumococcal polysaccharide vaccine. Both capsular polysaccharides S3 and S8 (Table 2; 4, 9) contain cellobiuronic acid in their repeating unit, a disaccharide consisting of d-GlcA $\beta(1\rightarrow 4)$ linked to d-Glc. In S3, the cellobiuronic acid units are $\beta(1\rightarrow 3)$ linked to each other (Reeves & Goebel 1941), while in S8 two additional α-linked hexoses are present, which all are connected in a $(1\rightarrow 4)$ linkage (Jones & Perry 1957).

The conformation of crystalline S3, studied by X-ray diffraction and stereochemical analysis (Marchessault et al. 1980), is a chain consisting of a threefold lefthanded helix, with a disaccharide unit of 0.923 nm (six monosaccharides in one turn). For S8, a twofold screw axis was proposed (Winter & Adelsky 1981).

Decrease in molecular mass of S3 ($\sim$ 220 kD) is accompanied by a reduction in its immunizing and tolerance inducing properties (Howard et al. 1971). S3 and S8 have been found to be degraded by specific endo-enzymes from *Bacillus palustris* strains isolated from soil (Dubos & Avery 1931). The $\beta(1\rightarrow 4)$ linkages of cellobiuronic acid in S3 are cleaved, with the most effective chain length for enzyme substrate interaction being more than 4 hexose units whereas by acid hydrolysis the $\beta(1\rightarrow 3)$ linkages are cleaved preferentially (Campbell & Pappenheimer 1966). S8-depolymerase has been shown to be a lyase, yielding tetrasaccharides with terminal unsaturated hex-4-enuronic acid residues (Becker & Pappenheimer 1966).

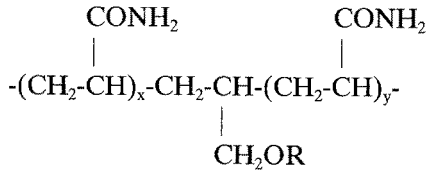
Types 3 and 8 anti-pneumococcal sera each contain a complex and heterogenous mixture of antibody molecules, whose specificity is determined by the different linkages and hexose units of S3 and S8. The size of the combining sites on antibody molecules have been studied by quantitative inhibition analysis of antigen-antibody interaction, using oligosaccharide determinants obtained by partial acid- or enzymic hydrolysis of S3 and S8 (Campbell & Pappenheimer 1966; Heidelberger & Kendall 1933; Mage & Kabat 1963). Up to 6–8 hexose units, the longer the chain length of the oligosaccharide, the more effective it becomes as an inhibitor of the homologous precipitin reaction. Cross-reactions are more readily inhibited than the homologous reaction. At least 6 hexose units are necessary in order to inhibit the homologous precipitin reaction in rabbit anti-S3 serum completely. Inhibition studies on different oligosaccharides of S3 indicate variation of the size of the combining regions of antibodies from different sera with a heterogeneity in specificity and suggest that the antigenic determinant of S3 occurs at intervals along the linear chain (Mage & Kabat 1963). Differences have been observed for hapten inhibition potency at differently acquired tetrasaccharides. Tetrasaccharides derived from acid hydrolysates have been reported to give a stronger inhibition than enzymically obtained tetrasaccharides. Labelled S3-hexasaccharides derived by reduction with NaB^3H_4 from acidic and enzymic hydrolysates have been used to study their interactions with rabbit and anti-S3 antibodies and their purified Fab fragments (Katz & Pappenheimer 1969). From this it was seen that the hexasaccharide derived from acid hydrolysates is 2–3 times more tightly bound than the hexasaccharide derived from enzymic hydrolysates, which is to be expected, since the carboxyl groups of the glucuronic acid residues account for a major fraction of the binding energies in both type 3 and 8 systems (Pappenheimer et al. 1968).

Circular dichroic spectra of homogenous anti-S3 antibodies show significant changes on binding of the hexasaccharide hapten (Holowka et al. 1972), which suggests changes in optical activity due to changes in the asymmetric environments of aromatic chromophores (tryptofan and tyrosine) in the combining site, and/or changes in orientation inside or beyond the site. Different antibodies show different spectra and different structures for the combining sites.

Cellobiuronic acid *p*-aminobenzyl glycoside, conjugated to horse serum globulin by diazotation,

gives rise in rabbits to antibodies which agglutinate type 3 pneumococci. Serum of rabbits immunized with the artificial cellobiuronic acid antigen is effective in protecting mice against infection with lethal doses of types 2, 3 and 8 pneumococci (Goebel 1939), though not of type 1.

S3 polysaccharide, conjugated to sheep erythrocytes (Paul et al. 1971), or BSA, Diphtheria toxoid and Tetanus toxoid by means of EDC (Beuvery et al. 1982), produces IgM and IgG antibodies in mice with a booster effect in the IgG response after a second dose of the protein conjugate. Memory B-cells can also be triggered to the production of IgG antibodies after a second injection with S3 alone. Hexasaccharide derived from a partial acid hydrolysate of S3 conjugated to different protein carriers (Snippe et al. 1983a), induce IgM antibodies and protection of mice from lethal challenge with type 3 pneumococci. Booster immunization gives rise to IgG antibodies. Hexasaccharide coupled to stearylamine and incorporated into a liposomal membrane also induces IgM and protection to a lethal dose of *S. pneumoniae* type 3 in mice, but no booster effect is observed (Snippe et al. 1983b). Two different synthetic disaccharide antigens (*A* and *B*), copolymerized with acrylamide, were tested for their serological specificity (Chernyak et al. 1985). Sequence *A* is a substantially stronger immunodeterminant than sequence *B*.



A R = β-D-Glc*p*A-(1→ 4)-β-D-Glc*p*-(1→
B R = β-D-Glc*p*-(1→ 3)-β-D-Glc*p*A-(1→

2. *Streptococcus pneumoniae type 4*

The pyruvyl group linked to O-2 and O-3 of D-Gal is a powerful immunodeterminant (Heidelberger et al. 1970) in type 4 capsular polysaccharide (Table 2; 5) (Jansson et al. 1981), and its removal gives rise to marked changes in immunological specificity (Higginbotham et al. 1970). Depyruvylated S4 resembles the pneumococcal C-substance immunologically (Heidelberger 1983), also in its reaction with CRP, which has been ascribed to the unmasking of GalNAc residues by removal of the pyruvic acid ketal ring.

The synthesis of β-D-Man*p*NAc-(1→ 3)-α-L-Fuc*p*NAc-(1→ 3)-D-GalNAc, forming part of the repeating unit of S4, has been described (Horito et al. 1986).

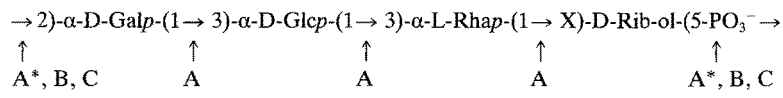
3. *Streptococcus pneumoniae type 5*

S. pneumoniae type 5 produces a capsular polysaccharide (Table 2; 6) with unusual sugar components (Jansson et al. 1985). The alkaline labile sugar component in S5 is 2-acetamido-2,6-dideoxy-D-xylo-hexos-4-ulose (Sug*p*)[b]. L-Pne*p*NAc stands for N-acetyl-pneumosamine or 2-acetamido-2,6-dideoxy-L-talose. Reduction of Sug*p* will produce a more stable polysaccharide, which will display a similar immunological activity. The long established immunological relationship between *S. pneumoniae* types 2 and 5 has tentatively been ascribed to the presence of GlcA residues (Heidelberger 1962).

S5 is non-immunogenic in adults, but provides a pathogenic potential to bacterial colonization of neonates (Denis et al. 1983).

4. *Streptococcus pneumoniae serogroup 6*

Group 6 organisms are among the more frequent causes of pneumococcal infections throughout the world at all ages, although they are common in pediatric patients. Immunological responsiveness of infants to group 6 pneumococci is delayed until the age of 4–6 years or more (Douglas et al. 1983). In adults, approximately 80% of disease isolates are of type 6A, and 20% of type 6B pneumococci (Robbins 1978), while in children most isolates are of type 6B. There are no significant differences between the antibody responses to type 6A and 6B polysaccharide vaccines in adult volunteers (Lee et al. 1981). The degree of cross-immunization varies



Sites of hydrolysis by:
A = acid, A* phosphodiesters are stable at pH 4, while monophosphate esters result in formation of inorganic phosphate.
B = alkali
C = hydrogenfluoride

X = 3, S6A
X = 4, S6B

Scheme 3.

between individuals (Braconier et al. 1983b), but increased opsonic activity towards type 6B was found in 75% of the patients after vaccination with S6A. Since there is concern about multiple antibiotic-resistant strains of pneumococcal type 6A (Applebaum et al. 1977), development of an effective vaccine against this type is especially important.

S6A and S6B (Table 2; 7, 8), which are among the least immunogenic of the polysaccharides in the multivalent pneumococcal vaccine, are polymers of identical repeating tetrasaccharide composition (Rebers & Heidelberger 1961; Kenne et al. 1979). The only structural difference lies in the linkage of rhamnose to ribitol, which is α-(1→ 3) in S6A, while S6B has a α(1→ 4) bond.

The α-D-Gal residue of the repeating unit has been regarded as immunodominant (Heidelberger & Rebers 1960) as concluded from inhibition experiments. The cross-reactions of certain polysaccharides in anti-S6 serum has been attributed to the presence of non-reducing end-groups of D-Gal. Group 6 polysaccharides contain only phosphate-linked D-Gal residues, which might function like an end group in antibody-producing serum. The phosphate free tetrasaccharide unit of S6A is a good inhibitor, but the phosphate monoester is a better one (Rebers et al. 1961). Of the three distinguished antigenic determinants, two are common to S6A and S6B (Lagergård & Branefors 1983). A minor part of the antibodies is directed against the common determinant D-RIB-OL-5-PO_4^-, WHICH IS RESPONSIBLE FOR CROSS-REACTIVITY WITH ANTI-*Haemophilus influenzae* type b sera. The determinant not in common must involve the different linkage of L-Rha to D-Rib-ol. Natural IgG antibodies with specificity for α(1→ 3) linked D-Gal residues, found in normal human sera (Galili et al. 1985), may be responsible for the poor immunogenicity of group 6 polysaccharides.

Conjugates of S6A polysaccharide to tetanustoxoid were tested for immunogenicity in juvenile and infant rhesus monkeys with disappointing results; only a few monkeys responded with protective levels of anti-S6A antibodies (Schneerson et al. 1984). In adult human volunteers the conjugate elicited only a small increase in specific antibodies (Schneerson et al. 1986).

Oligosaccharides derived from S6A capsular polysaccharide have been coupled to a protein carrier and reported to show a high immunogenicity of the glycoconjugate with specificity for the carrier protein and the type 6A pneumococcal capsular polysaccharide (Porro et al. 1985). The oligosaccharides obtained by gradual acid hydrolysis with the dimensions of an octasaccharide, were conjugated directly to a protein, using derivatives reductively aminated with $NaCNBH_3$ and NH_4Cl, which were subsequently activated with disuccinimidyl ester of adipic acid for coupling to protein. This reaction can be performed only with reducing oligosaccharides obtained by the less specific mild acid hydrolysis method. For oligosaccharides of alkaline degraded polymers, which gives specific splitting of the phosphodiester bond (Scheme 3) (Zon et al. 1982; Van Dam et al. 1989a), other coupling procedures are necessary, because no reducing-end groups are released.

The trisaccharide fragment α-D-Gal*p*-(1→3)-α-

D-Glc*p*-(1→3)-L-Rha*p* of S6AB has been synthesized recently (Slaghek et al. 1988).

Antibodies of the IgM class induced in mice by pneumococcal capsular polysaccharides of types 6 and 19 have been reported to be specific for contaminant pneumococcal cell wall polysaccharide (Fairchild et al. 1986).

5. *Streptococcus pneumoniae serogroup 9*

S. pneumoniae serogroup 9 contains four capsular types 9N, 9A, 9L, and 9V, with related polysaccharides (Table 2; 10–13), which account for 5.8% of all bacteremic and 3.7% of all meningeal pneumococcal infections. Types 9N (34%) and 9V (57%) (Table 1) predominate in pneumococcal disease, but the prevalence of the types varies according to age, geographical location and the interval studied (Robbins et al. 1983; Szu et al. 1981, 1982). Type 9N infections are more prevalent in adults, while type 9V is the most common cause of disease among young children. Both S9N and S9V are included in the 23-valent capsular polysaccharide vaccine. The GlcA residue, present in all four types, is an important immunological determinant. Type 9A antiserum showed the most extensive cross-reactions with the four group 9 polysaccharides. Inhibition of the binding of S9N to PMN's by prior treatment with S9V is strong, while the reversal gives low inhibition (Lu et al. 1987). The high degree of inhibition of binding of S9V is not directly related to the production of cross-reactive antibodies to S9N. The serum antibody and PFC responses in mice immunized with S9V were significantly greater than responses to S9N.

Binding of S9N and S9V to human lymphocytes and PMN's, is temperature-dependent and type-specific. For S9V each PMN has 12×10^4 binding sites (affinity constant $8.5 \times 10^8\ M^{-1}$), while 7.8×10^4 binding sites (affinity constant $1.6 \times 10^8\ M^{-1}$) have been found for S9N (Lu et al. 1987). Each lymphocyte has 6.4×10^4 binding sites for S9N ($3.7 \times 10^8\ M^{-1}$) and 9.5×10^4 binding sites for S9V ($9.4 \times 10^8\ M^{-1}$).

Trisaccharides
β-D-ManNAc*p*-(1 → 4)-α-D-Glc*p*(1 → 2)-D-Glc*p* and β-D-ManNAc*p*-(1 → 4)-β-D-Glc*p*(1 → 2)-D-Glc*p* which form part of S9A and S9V, respectively, have been synthesized recently (Paulsen et al. 1988).

6. *Streptococcus pneumoniae serogroup 12*

Infections due to group 12 rarely occur (< 1%) in the pediatric population (Austrian 1981). Immunological evidence (Goodman & Kabat 1960; Suzuki & Hehre 1964) suggests that kojibiose (α-D-Glc (1 → 2)-D-Glc) is an important determinant of S12F. S12F has been conjugated to diphtheria toxoid with SPDP via its ManNAc-cysteamine derivative. The conjugate elicited antibodies in young mice and had TD properties as it gave booster responses (Fattom et al. 1988). S12A has significant structural features in common (Table 2: 20, 21) (Leontein et al. 1981, 1983), which accounts for its serological reactions (Lund 1970).

7. *Streptococcus pneumoniae type 14*

Type 14 pneumococcal polysaccharide (Table 2: 23) (Lindberg et al. 1977), which is structurally identical with the core antigen of group B *Streptococcus* type III (GBS III) (Jennings et al. 1980b; Wessels et al. 1987), is a rare example of a neutral polysaccharide capsule involved in human bacterial disease. The capsular polysaccharide of GBS III differs from S14 only in that it has a α(2→ 3) linked terminal sialic acid residue attached to the branching β-Gal, which is not immunodominant (Kasper et al. 1978). Early investigations showed extensive cross-reactivity of anti-S14 serum with a wide variety of carbohydrate antigens (Heidelberger 1960) (e.g. the core oligosaccharide moiety of the R-type lipopolysaccharide of *Neisseria gonorrhoeae,* which contains non-reducing D-Gal and D-GlcNAc, Diena et al. 1979), in which the non-reducing β-Gal residues were considered to be the principal antigenic determinant (Kabat 1962). The affinity of antibody binding to S14 derived oligosaccharides, prepared with an endo-β-galactosidase from *Cytophaga keratolytica,* increased as the

chain length of the antigen increased, indicating a role for conformational immunodeterminants on S14 (Wessels & Kasper, 1989).

Both complement pathways participate in the opsonization of type 14 pneumococci, in which specific antibodies play an important role (Gardner et al. 1982). Antisera directed against S14 display opsonic activity against GBS III (Fischer et al. 1978, 1979), and could be functional in the production of antibodies against GBS III. Like the capsules of the most prevalent *Escherichia coli* types K1 and K5 and the group B capsule of *Neisseria meningitidis,* S14 is cross-reactive with human tissues and thus poorly immunogenic. Production of antibodies to these determinants is highly undesirable and consequently is suppressed by the immune system. Rabbits immunized with GBS III or S14 vaccines develop cold agglutinin- or auto-antibodies (Colling et al. 1983). Horse anti-S14 sera are known to agglutinate human erythrocytes resembling cold agglutinin sera with anti-I specificity, reacting more strongly with adult erythrocytes (I) than cord erythrocytes (i). These anti-S14 sera react with the repeating *N*-acetyl-lactosamine structures [→ 3)-β-D-Gal*p*-(1→ 4)-β-D-GlcNAc*p*-(1→] found in i and I antigens (Kabat et al. 1978; Pennington & Feizi 1982). This reaction is stronger with *N*-acetyl-lactosamine linked β (1→ 3) to Gal occurring in both i and I determinants, than with β (1→ 6) linked residues, which occur as branching point in the I determinant only.

In this context it is interesting to note, that attaching pneumococci specifically bind to glycoconjugate receptors (Andersson et al. 1983a) containing the disaccharide β-D-GlcNAc*p*-(1→ 3)β-D-Gal*p*, which (substituted at various positions) is part of many saccharide chains active as blood-group antigens (ABH, Lewis and I/i antigens, Watkins 1980), but is also part of S14. An endo-β-galactosidase, which hydrolyses β-D-Gal*p*-(1→ 4) β-D-GlcNAc*p* only of unbranched lactosamine glycan-chains, was isolated from the culture supernatant of *S. pneumoniae* (Fukuda 1985). The natural presence of neolacto- and lactotetraose in human milk and colostrum interferes with pneumococcal colonization in the newborn (Andersson et al. 1985). After the age of 5 to 6 years, at which the ABH bloodgroup isoantigens reach their adult level, infection with type 14 organisms declines.

The reactivity of anti-S14 serum with melibiose (α-D-Gal*p*-(1→ 6)-β-D-Glc) (Colling et al. 1983) indicates a possible important role for the β-D-Glc*p*-(1→ 6)β-D-GlcNA*p* moiety in the antigenic determinant, which is found solely in S14, GBS III and some other pneumococcal capsular types of groups 11 and 15.

Synthesis of the repeating tetrasaccharide structure of S14 has been achieved (Zurabyan et al. 1976; Amvam-Zollo & Sinaÿ 1986), and synthesis of S14 polysaccharide has been recently reported (Kochetkov et al. 1987), although no reports have been made of the immunogenicity of these derivatives. Tetrasaccharides obtained by deamination of S14, coupled to stearylamine and incorporated into a liposomal membrane, induce a slow raise of specific anti-S14 IgM antibodies (Van Dam et al. 1989b) in mice.

8. Streptococcus pneumoniae serogroup 15

The same pentasaccharide unit is synthesized by all types of *S. pneumoniae* group 15, but there are two different routes for the polymerization of this unit (Jansson et al. 1987). S15F and S15A contain the same sequence of sugars in a linear arrangement (Perry et al. 1982; Carroff & Perry 1984), whereas S15B and S15C have a branched structure (Table 2; 24–27), with a core antigen identical to S14. As expected, S15B gives a strong cross-reaction in anti-S14 sera, but S15C only gives rise to a mild reaction (Heidelberger 1983). S15C and S15B are identical, save for the presence of an O-acetyl moiety in the latter. The simultaneous presence of types 15B and 15C in aspirates from the middle ears of children with otitis media has been observed (Venkateswaran et al. 1983). The phenomenon of cells of type 15B arising from a type 15C strain has been ascribed to labile inversion of a DNA segment.

9. Streptococcus pneumoniae serogroup 18

S. pneumoniae serogroup 18 consists of four differ-

ent types, which elaborate capsular polysaccharides of related structures (Table 2; 30–32). The structures of S18F, S18A, and S18C have been established (Lugowski & Jennings 1984; Jansson et al. 1988a, b), and are built up from branched pentasaccharide repeating units, containing glycerolphosphate.

Removal of *O*-acetyl groups in S18F abolishes its immunological specificity (Estrada-Parra & Heidelberger 1963). The structures are not in agreement with the strong inhibition of isomaltose in the S18-anti-S18 system. The presence of similar arranged trisaccharides β-L-Rha*p*-(1→ 4)-β-D-Glc*p*-(1→ 2)-β-D-Gal*p* in both S18F and S23F, is responsible for cross-reactions of S18F in anti-S23F sera (Heidelberger 1983).

10. Streptococcus pneumoniae serogroup 19

Almost all infections caused by group 19 pneumococci are of types 19A and 19F, and are found in all age groups (Robbins et al. 1983). Group 19 capsular polysaccharides have been reported to be poor immunogens in children under 2 years, perhaps because of their chemical instability.

The same repeating trisaccharide structure has been proposed for *S. pneumoniae* type 19F and 19A capsular polysaccharides (Table 2; 33–35) (Lee & Fraser 1980; Ohno et al. 1980; Katzenellenbogen & Jennings 1983), in which only the linkage of the D-Glc to L-Rha is different. In S19F (33) this is an α(1→ 2) linkage, while it is reported to be an α(1→ 3) linkage in S19A (34). A contradictory structure for S19A has been published (35), built up from the same repeating unit as S19F but with two additional side chains attached at C-2 of Glc and C-3 of Rha (Lee & Fraser 1980). The differences between these structures have been ascribed to culture conditions (Lee et al. 1987a). The presence of a glycosidically linked phosphodiester bond accounts for the unstability of group 19 polysaccharides towards mild acid or alkali.

The repeating trisaccharide units β-D-ManNAc*p*-(1→ 4)-α-D-Glc*p*-(1→ 2)-α-L-Rha*p* of S19F and β-D-ManNAc*p*-(1→ 4)-α-D-Glc*p*-(1→ 3)-α-L-Rha*p* of S19A have recently been synthesized (Panza et al. 1987, 1988; Sugawara & Igarashi 1988; Paulsen et al. 1988).

Extensive cross-reactions have been observed with certain *Klebsiella* capsular polysaccharides (e.g. types K2, K9, K32, K47, and K48). These polysaccharides induce antibodies reactive with S19F and cross-react with equine S19F antisera, but not with rabbit antisera. Higher immunological responses to S19F were induced in mice injected with both S19F and K2 or K47 (Lee & Wang 1985). The repeating oligosaccharide structures of *Klebsiella* capsular polysaccharides show different similarities with S19F (Gahan et al. 1967; Lindberg et al. 1972; Bebault et al. 1978; Björndahl et al. 1973). In K9, K32 and K47 the →2)-α-L-Rha*p* residues must account for the observed cross-reactivity, while in K2 the →4)β-D-Man*p*-(1→ 4)-α-D-Glc*p* residues will contribute. Also important is probably the presence of the acidic groups in similar spatial orientation.

Polysaccharide conjugates of S19F with different protein carriers induce higher IgM and IgG2 antibody titres in young mice (Lin & Lee 1982; Lee et al. 1987b). Maternal immunization with S19F or S19F-protein conjugate, before or during gestation and/or lactation, induces a significantly stronger response in offspring.

11. Streptococcus pneumoniae type 23

Type 23F pneumococci accounts for almost all group 23 isolates from patients of all ages. The capsular polysaccharide of type 23F consists of a phosphated tetrasaccharide repeating unit with α-L-Rha side-chains (Table 2; 38) (Richards & Perry 1988), which are immunodominant, as was seen from cross reactions with certain *Klebsiella* (e.g. K17, K19, K47, K56, K64, and possibly also K14) and Streptococcal strains (Heidelberger & Nimmich 1976). Synthesis of phosphated tri- and tetrasaccharide fragments of S23F have been reported (Van Steijn et al. 1989).

VI. Alternative strategies

Continuing efforts at preventing pneumococcal disease are imperative. Pneumococcal pneumonia is an increasingly important disorder among the elderly. The high rate of invasive pneumococcal disease and the high mortality associated with pneumococcal pneumonia (30–40%) emphasize the need for an effective vaccine (Murphy & Fine 1984). Protection against pneumococcal infection is primarily dependent on antibodies, complement and proper functioning of phagocytic cells. There is little that can be done to alter the levels or the function of the latter two host defense factors (Schiffman 1983a). In almost all host defense systems antibodies are the limiting factor (Schiffman 1983b), while in addition to the antibody titres functional activity also is an important determinant for the efficacy of antibodies in host resistance. Alternative approaches for vaccine development against encapsulated bacteria are summarized in the next paragraph.

Subcellular vaccines

Subcellular vaccines prepared from ribosomal (RNA) fractions of *S. pneumoniae* give specific protection to mice against homologous challenge, which is exclusively a humoral response (Robert et al. 1982). The efficacy of ribosomal vaccines has been associated with the presence of membranous or cell wall components (Au & Eisenstein 1981b).

Immunoglobulins

Passive immunization with immunoglobulin preparations may trigger antibody-dependent responsiveness in immunocompromized hosts.

1. Hyperimmunoglobulin prepared from the plasma of immunized donors has been used for passive antibody prophylaxis (Siber et al. 1984). Besides prevention of pneumococcal bacteremia in mice, human immunoglobulin preparations also are effective in treatment of mice infected with pneumococcal types 3 or 7F (Chudwin 1989).
   IgG infusions in children (1–4 years) increase specific IgG antibody concentrations, but subsequent infusions resulted in a decrease (Prellner et al. 1986). Otitis media may be prevented by immunoglobulins, but may also inhibit development of immunity in children (Shurin et al. 1988). In commercially available immunoglobulin preparations, low levels against types 3, 8, and 18C were recorded (Offenbartl et al. 1986). Those preparations showed good protection in splenectomized rats against type 1 pneumococcal sepsis. In pregnant women given immunoglobulin, transplacental passage of IgG occurs from the 34th week of gestation (Morell et al. 1986).
2. A monoclonal IgA,$\varkappa$ antibody specific for S8 is produced by an Epstein-Barr virus immortalized cell line of human lymphocytes, which does not fix complement but induces direct killing of type 8 pneumococci in vitro and opsonization by mouse macrophages (Steinitz et al. 1986). Such monoclonal antibodies are suitable for use in therapy, provided that they improve the recipient's immunity against the pathogen.
3. Monoclonal antibodies specific for a surface protein (protein A) of the unencapsulated. *S. pneumoniae* strain R36a react with 6 of the 21 encapsulated and unencapsulated pneumococcal strains tested, and protect mice from infection with certain type 2 and type 3 strains (McDaniel et al. 1986). There is no correlation with capsular typing.
4. Anti-idiotypic (Ab2) and anti-anti-idiotypic (Ab3) responses to various antigens (including polysaccharide antigens) provide a mechanism for the production of antibodies specifically reacting with a given antigen in hosts which have never been stimulated with the antigen. Ab2 formed on immunization with antibody (Ab1) to a given antigen may show properties of original antigenic determinants (Jerne et al. 1982), and immunization with Ab2 may elicit formation of antibodies (Ab3), which will react with the original antigen, as does Ab1 (Urbain et al. 1983).

The idiotype may prime newborns to respond to subsequent injection with antigen, and may overcome the failure of infants to respond to bacterial polysaccharides. Peptide mimics of polysaccharide epitopes may form the basis of a new generation of vaccines.

5. Monoclonal anti-idiotype antibody coupled to a protein carrier has been used as a vaccine against *S. pneumoniae* infection in mice. A high-titre anti-phosphorylcholine antibody developed, increasing the resistance to bacterial challenge (McNamara et al. 1984).

Polysaccharide-protein conjugates

Increasing immunogenicity of carbohydrate epitopes is achieved by covalent attachment to a carrier protein. The protein-conjugated carbohydrates are converted from a TI to a TD immunogen, resulting in a significant increase in immunogenicity (Paul et al. 1971; Braley-Mullen 1974; Beuvery et al. 1982; Schneerson et al. 1984; Marburg et al. 1986; Lee et al. 1987; Fattom et al. 1988). The importance of the carrier protein for a hapten is its ability to stimulate a T_H-cell response. Polysaccharide-protein conjugate vaccines need to contain domains that interact with T_H-cells and the histocompatibility (MHC) Ia antigens (Bialy 1987) before a potent vaccine is realized. The relative molecular mass of the carrier protein is unimportant, although the larger the protein, the more likely it is to contain helper determinants for a greater number of MHC types. Antibody production, however, is often switched from IgG2 to other isotypes less effective in affording protection (Moshier et al. 1987), and immunization with polysaccharide-protein conjugates may not prime for subsequent responses to the polysaccharide alone (Insell & Anderson 1986). The demonstrated vaccine efficacy of *Haemophilus influenzae* type b capsular polysaccharide coupled to diphtheria toxoid in infants (Eskola et al. 1987), may have immediate implications for vaccine development.

Oligosaccharide-carrier vaccines

Oligosaccharides derived from capsular polysaccharides coupled to protein carriers (Snippe et al. 1983a, Porro et al. 1985), give rise to similar improvement of immunogenicity as polysaccharide-protein conjugates, but might be more likely to engage a T_H-cell effect than conjugates made with the corresponding polymer (Paul et al. 1971). The size of the determinants may vary with capsular type. For *Haemophilus influenzae* type b capsular polysaccharide, it has been shown that there is a minimum size of conjugate required for immunogenicity (Anderson 1983). Determinants at the non-reducing end of linear carbohydrates are found once per molecule.

Oligopeptides recognized by T_H-cells must have an amphipatic structure (DeLisi & Berzofsky 1985), of which the hydrophilic portion of the epitope seems to be recognized by T-cell receptors. The hydrophobic portion binds the MHC antigen at the surface of the antigen-presenting cell, or attaches the epitope to its plasma membrane (Babbitt et al. 1985; Ashwell & Schwartz 1986; Watts et al. 1986). Liposomes carrying antigens can substitute for antigen-presenting cells to induce antigen-specific MHC-restricted interactions with lymphoid cells (Walden et al. 1985), when they contain class II MHC molecules (antigen-presenting liposomes). If oligosaccharide epitopes, modified by coupling to lipids adopt such a structure, remains to be proven. Haptenated liposomes carrying oligosaccharide epitopes of *S. pneumoniae* types 3 and 14 have been shown to be immunogenic in mice (Snippe et al. 1983b; Van Dam 1989b), although no TD antigen is obtained. Stearyl-isomaltosyl oligosaccharide induced proliferation of pre-existing clones with sites complementary to oligosaccharides larger than those present in the immunizing antigen (Lai et al. 1985). The immunogenicity of such neoglycolipids should be studied in more detail.

Immunoadjuvants

Immunoadjuvants are defined as compounds which are added to antigens or vaccines with the intent of stimulating the immune response so that protection is improved, a lesser quantity of antigen is required and fewer doses are needed to be given (WHO technical report 1976). The chemical nature of these so-called immunomodifiers varies from rather simple (chemical well-defined) compounds to extremely complex (poorly-defined) substances (Borek 1977; Whitehouse 1977).

Addition of immunoadjuvants to polysaccharide antigens (TI-antigens) are aimed at expanding the antibody response to IgG isotypes with greater protective capacity (Warren et al. 1986) and eventually induction of immunological memory. With respect to S3 it was observed that monophosphoryl lipid A as well as trehalose dimycolate increase the magnitude of the IgM antibody response and these immunoadjuvants were also able to evoke antibodies of the IgG1 and IgG3 classes (Baker et al. 1988a,b). These results are explained by elimination by the adjuvants of the inhibitory effects of T_S-cells without altering the T_H- or T_A-cell function.

The usefulness of different non-ionic blockpolymers (NBP's) as adjuvants was studied extensively recently (Zigterman et al. 1988, 1989; Van Dam et al. 1989a,b). These NBP's were tested on their ability to increase the immune response to oligosaccharide-protein and -lipid conjugates. Against oligosaccharide-protein conjugates the antigen specific IgM and IgG antibody responses are increased by NBP's in both normal mice as well as in newborn Xid mice. These antibodies show an increased affinity towards the antigen and a balanced distribution of the IgG antibodies over the four subclasses was observed. A single injection of conjugate and NBP resulted in a long-lasting protection to a lethal dose of *S. pneumoniae* type 3 (Zigterman et al. 1989). Moreover newborn mice were also protected by specific antibodies. The results are due to observed interactions between NBP's and the antigen, which result in altered antigen distribution, and interactions between NBP's and macrophages. Similar observations for *S. pneumoniae* type 14 were made recently by using the adjuvant Quil A (Verheul et al. 1989).

The combination of poly- or oligosaccharide-protein conjugates with new developed immunoadjuvants are promising candidates for a future pneumococcal vaccine, which will also be effective in young children.

Chemically modified polysaccharides

Improvement of immunogenicity has been achieved with *Neisseria meningitidis* group B capsular polysaccharide by a simple chemical modification (substitution of *N*-propionyl for the *N*-acetyl groups) (Jennings et al. 1986, 1987). Many unsuccessful attempts to increase the immunogenicity of capsular polysaccharides included the addition of adjuvants, acylation and covalent attachment of muramyl dipeptides (Schneerson et al. 1987). However, enhancement of anti-polysaccharide responses in neonates may be achieved by coupling of small mitogenic moieties (like lipid A), which converts the TI-2 antigen into a TI-1 antigen (Moshier et al. 1987), capable of activating immature B-cells. Other modifications of carbohydrate determinants might lead to interactions with Ia antigens and T_H-cells.

VII. Concluding remarks

From the previous chapters it can be concluded that our knowledge of the pneumococcus, which is still one of the most serious causative agents of infectious diseases in man, has been expanded considerably the past decades. There is some insight in the way the pneumococcus invades the host. Much is known of the response of the immune system to the infecting agent and which factors are important for pneumococci to resist the host defences. There are indications of which conditions of the host predispose to infection and how resistance against pneumococcal disease may be improved by vaccination. Although much progress has been made, there are still many unresolved questions, which

require more detailed research in order to obtain insight in the pathogenicity of the pneumococcus and possibilities for efficient disease control.

It has been possible to formulate the polyvalent pneumococcal vaccine, which is effective in inducing long-term persisting specific antibodies in normal subjects, but generally not in the high-risk groups for pneumococcal infections. The need for a highly effective vaccine against pneumococcal diseases is of great importance for these groups.

Improved vaccines to *Streptococcus pneumoniae* should be approached from different sites, depending on the underlying condition of the recipient. One approach is improvement of the immunogenicity of the pneumococcal polysaccharide antigens, by covalently coupling of the polysaccharide or a derived oligosaccharide epitope to a T_H-stimulating carrier. In this approach there are promising posibilities for synthetic oligosaccharide epitopes, which can be more easily adapted to give immunodeterminant epitopes than isolated oligosaccharides from the native polysaccharides. Moreover, the coupling to carriers of synthetic oligosaccharides may be better controlled and more specific. Immunological adjuvants may be of great importance in augmenting preferentially the IgG antibody production to these neoglycoconjugate-antigens.

Given the great number of carbohydrate antigens involved it is recommended to limit the number of improved antigens to the most urgent serotypes responsible for infections in infancy (e.g. types 6AB, 14, 19F, 23F). Revaccination with the pneumococcal polysaccharide vaccine may be considered at an older age (8–10 years), when children are capable of a better response to polysaccharide antigens.

Besides vaccination with polysaccharide-derived antigens, which trigger the immune system of the recipients, passive immunization with immunoglobulin preparations should be considered in immunocompromized hosts.

Besides the threat of disease and death and the suffering caused by pneumococci in the high-risk groups and the inconvenience for relatives and friends, also the high economic costs, which are involved in hospitalization and medical treatment, should be considered as arguments for the development of effective vaccines.

However, an unfortunate side effect of vaccination resulting in life long protection against pneumococcal disease, may be that the elderly are denied dying a relative mild death from pneumococcal pneumonia, aptly called by Sir William Osler 'the friend of the aged', bringing peace to those who suffer.

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