LIPOSOMAL VACCINE TO *STREPTOCOCCUS PNEUMONIAE* TYPE 3 AND 14

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INTRODUCTION

*Streptococcus pneumoniae* is responsible for lower-respiratory-tract infections in humans and also the most common cause of otitis media (bacterial middle ear infection) in children. Pneumococcal pneumonia is rarely a primary infection of the lung but such factors as damage to the respiratory tract, fatigue, chilling of the body and general debilitation predispose to infection. *S. pneumoniae* can be isolated from the pharynx of 30-70% of apparently normal humans.

The first isolations of the pneumococcus were done independently by Pasteur and Sternberg in 1881. Pneumococci are ovoid, non-motile, non-sporing organisms which usually occur in pairs (old nomenclature: *Diplococcus pneumoniae*) enveloped by a capsule. The capsule is the most striking morphologic feature of the organism and the most voluminous when the organism is at its most virulent stage. *Streptococcus pneumoniae* is divided into types that are characterized by these carbohydrate capsules which differ in chemical composition and structure (Larm and Lindberg, 1976; Kenne and Lindberg, 1983; Jennings, 1983). There are 85 different capsular types of the pneumococcus and two systems of nomenclature: the Danish, now generally used, and Eddy's, which was in use in the United States (Kaufman et al, 1960).

The capsular polysaccharides play an important role in infection. They render the bacteria resistant to non-specific host defence. Under unfavourable growth conditions, the organisms become avirulent and lose their capsules and immunological specificity. Structural properties must account for the role of polysaccharide capsules in bacterial pathogenesis. Heavily encapsulated type 3 pneumococci are extremely virulent in mice, while type 37 pneumococci, having the same degree of encapsulation, are not. On the other hand, type 12 pneumococci, which have very small capsules, are extremely virulent in humans (Austrian, 1981).

The organism possesses no endotoxin but produces disease and ultimately death through its capacity to multiply in the tissues. The high rate of mortality from *S. pneumoniae* infection despite the availability of appropriate antibiotic therapy (with about 5% case fatality rate, which is even higher among patients with bacteremia and meningitis; 20-40%, Health and Public Policy Committee, 1986), prompted for preventive approaches.

One approach to obtain a carbohydrate vaccine is the isolation of capsular polysaccharides from a culture of living bacteria. Early successful experiments with the type specific capsular polysaccharides of Streptococcus pneumoniae as a vaccine were overshadowed by the discovery of sulfonamides and penicillin. Renewed interest for preventive vaccination was due to increasing incidence of antibiotic resistant bacterial strains (Finland, 1978). The prophylaxis of human infections with isolated polysaccharides from encapsulated bacteria has been the subject of profound and expanding research. Besides immunological experiments on animals and clinical trials with human volunteers, the chemical structure of capsular antigens of many pathogens were elucidated.

A polysaccharide vaccine that is built up of the capsular polysaccharide from 14 serotypes (Robbins, 1978; Austrian, 1981) has been licensed (Pneumovax®). Out of the 85 known pneumococcal serotypes these are the causative agents for 70-80% of the pneumococcal infections in the United States. A 23 valent vaccine that will cover nearly 90% of bacteremic infections in the U.S. was introduced in 1983 (Robbins, 1983). Epidemiological studies in different geographical areas showed some variation in prevalence of serotypes (e.g. serotypes 45 and 46 in South Africa and Asia) which necessitates an adaptation of the composition of a vaccine per region.

Although many positive reports have been published and pneumococcal vaccination is recommended for patients with a high risk for pneumonia (Finland, 1978) the effect of the vaccine on these groups and small children is often still unsatisfactory, due to the inherent immunological character of polysaccharide antigens. Moreover, it has not been possible to demonstrate any efficacy of the pneumococcal vaccine in preventing pneumonia in high-risk patients.

**Immune Response**

The (human) immune response to infections caused by encapsulated bacteria consists of a highly complex interplay of different cells and molecular compounds. Three factors play a role in the process of eliminating invading encapsulated bacteria, leading to ingestion and killing of the invading organisms: 1) phagocytosis by macrophages and granulocytes, which adhere either directly to microbes or via a mechanism mediated by the complement system or by specific antibodies, leading to ingestion and killing of the invading organisms; 2) activation of the complement system; 3) production of antibodies. In the early acute stages of infections the humoral immune response is the most important factor. Antibodies or immunoglobulins are produced by the interaction of two types of lymphocytes. For most antigens, thymus (T) derived lymphocytes stimulate the bone-marrow derived B-cells to produce specific antibodies of immunoglobulin type G (IgG), but polysaccharides are T-cell independent (TI) antigens, which activate B-cells directly to produce mainly IgM antibodies upon immunization. This TI-character of carbohydrates is a disadvantage of the existing polysaccharide vaccines. Moreover the immune response against bacterial polysaccharides is only developing in later stages of the ontogenesis, with consequently limited use for capsular polysaccharide vaccines in children. TI-antigens do not lead to "memory" induction which is necessary for a long lasting protection. When helper T-cells can be induced, which in turn stimulate B-cells to produce IgG antibodies (thymus dependent, TD-response) durable protection should be obtained. Conjugation of carbohydrates to protein as a macro-molecular carrier converts the antigen from a TI- into a TD-immunogen and, as a consequence, results in a significant increase in immunogenicity.