es a low level of antibodies measurable by standard techniques, and also induces $CD4^+$ and $CD8^+$ T cell responses.

In the renal transplant patients, vaccination was followed by a period of immunosuppression at the time of surgery. Although latent, natural CMV reactivates frequently in this situation, the Towne vaccine virus did not, and indeed replication of the Towne virus was not detectable except temporarily at the injection site (*15*). The induction of antibodies by vaccination to nonvirion antigens that are expressed only during viral replication, and the failure of inactivated Towne virus to immunize, provide evidence that replication of the attenuated strain is needed for the immune response.

Tests of vaccine efficacy have been performed in three ways. First, an unattenuated low-passage CMV was used to challenge 30 healthy volunteers who were CMV-susceptible, CMV-immune, or vaccinated with the Towne virus. In susceptible individuals, 10 plaque-forming units (PFU) of the challenge virus produced an infectious mononucleosis syndrome, whereas among the naturally immune, symptoms developed only in those volunteers receiving 1000 PFU of virus. Vaccinees were completely protected against a 10 PFU challenge, but at 100 PFU ~50% became infected, albeit with little or no symptoms (*15*).

A second test of vaccine efficacy consisted of three double-blind placebo-controlled studies of CMV-seronegative kidney transplant patients who received kidneys from seropositive donors. In all three studies, vaccination did not affect the incidence of CMV infection, but did reduce the occurrence of severe illness by 80 to 100% (*15*).

Finally, the vaccine was tested in mothers of children in day-care centers, who have high rates of contact-acquired CMV infection. Towne vaccination did not affect the rate of infection (*17*), although naturally immune women who had a significantly higher titer of neutralizing antibodies were resistant to infection.

Meanwhile, efforts to develop CMV vaccines that are based on newer technologies are also burgeoning. The entire viral genome has been sequenced and the envelope glycoproteins that induce neutralizing antibodies have been identified, as have the antigens that induce CTLs. The main candidate for a subunit vaccine is an envelope glycoprotein known as UL55 or gB, which is synthesized as a 130- to 140-kD precursor that is cleaved to proteins of 116 and 55 to 58 kD (*18*). Several domains, notably the immunodominant epitope on the 55- to 58-kD protein, account for most of the neutralizing antibodies in serum from convalescent patients, and purified gB has been shown to induce neutralizing antibodies and lymphocyte sensitization in both animals and humans (*19*, *20*).

The gene encoding gB has been inserted into several different vectors. Baculovirus-produced gB, in combination with the adjuvant QS21, is a potent immunogen in mice. Substantial quantities of gB can now be produced in animal cell culture (*21*). The gB gene has also been inserted into a deletion mutant of adenovirus type 5, and this replication-competent virus was shown to be immunogenic in small animals (*22*). A poxvirus from canaries, which gives only an abortive replication in mammals, is also being tested as a vector for gB. Phase I clinical trials of some of these candidates are being planned.

Other candidates for a CMV subunit vaccine include the gH glycoprotein, which also carries neutralizing epitopes. The gH glycoprotein is difficult to produce in vitro because it requires a chaperone (*23*), but antibodies against it may complement those induced by gB. Internal viral proteins such as the immediate-early proteins or matrix proteins may be the best inducers of CTLs. The importance of CMV-specific CTLs in transplant recipients is well known [for example, see (*24*)]. Thus, complex subunit or vectored vaccines may be needed to elicit good T cell responses. Other possible approaches to CMV vaccines include synthesis of peptides that mimic key epitopes, production of anti-idiotypic antibodies, and genetic manipulation of the Towne attenuated virus to augment its immunogenicity.

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Pneumococcal Disease: Prospects for a New Generation of Vaccines

George R. Siber

During the early 1900s, pneumococcal pneumonia was a much feared disease with a high mortality rate. The advent of effective antibiotics provided a new means to treat the disease, and as a result, early vaccine efforts were abandoned.

Nevertheless, *Streptococcus pneumoniae* (pneumococcus) remains the most common cause of bacterial pneumonia in the United States today. Disease rates are particularly high in young children, in the elderly, and in patients with predisposing conditions such as asplenia, chronic medical conditions (heart, lung and kidney disease, diabetes, alcoholism) or immunosuppressive illnesses, particularly AIDS (*1*). These same groups are at greater risk of pneumococcal spread to the bloodstream and to the central nervous system (meninges), and thus have a greater risk of death. In countries like the United States, which have introduced universal immunization against *Haemophilus influenzae* b (Hib), the pneumococcus is the most common cause of bacterial meningitis. On a global level, the pneumococcus is believed to be the most common bacterial cause of acute respiratory infections, which are estimated to result in more than 1 million childhood deaths each year (*2*). It is also associated with middle ear infections and sinusitis, which, though less severe illnesses, nevertheless incur substantial medical costs (*3*).

Recently, antibiotic-resistant strains of pneumococcus have emerged throughout

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the world. A recent survey of 13 U.S. hospitals in 12 states revealed that 6.6% of pneumococcal isolates were resistant to penicillin (*4*). In some hospitals, up to 20% of pneumococcal isolates are penicillin-resistant and a portion of these are also resistant to other antibiotics including third-generation cephalosporins (*5*). This alarming development underscores the need for more effective preventive strategies.

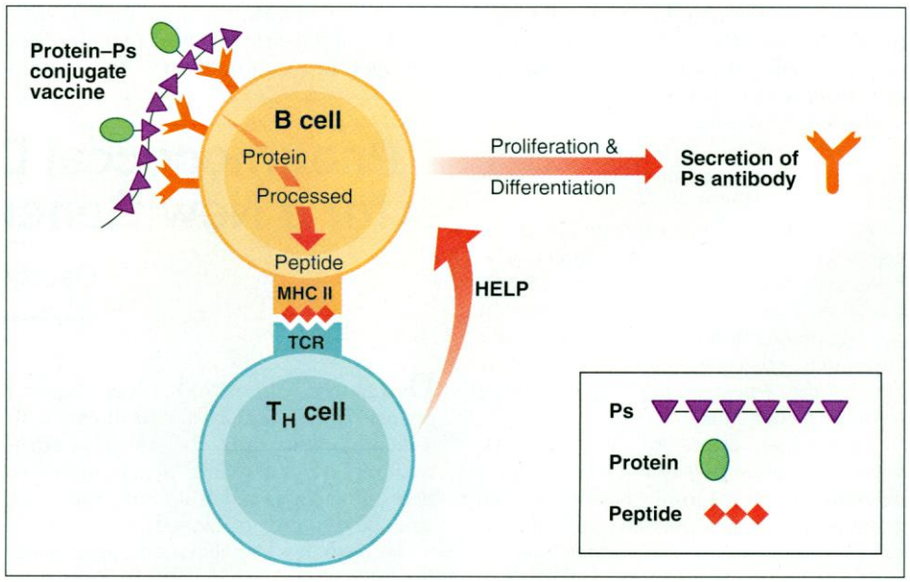
Like other encapsulated bacteria, the pneumococcus relies on its polysaccharide surface coat or capsule to evade the host's phagocytic defenses. Induction of anti-capsular antibodies by active or passive immunization has long been known to protect against disease by enhancing phagocytosis of the bacteria (*6*). However, the development of a vaccine with adequate coverage of pneumococci was complicated by the existence of at least 84 distinct pneumococcal capsular polysaccharide serotypes. This obstacle was overcome with the introduction of a polyvalent pneumococcal vaccine through the persistent efforts of R. Austrian and his colleagues and by researchers at Merck, Sharp, and Dohme. In 1977, a vaccine containing the purified polysaccharides of 14 of the most prevalent types was licensed; this vaccine provided coverage against ~80% of invasive pneumococcal isolates in the United States. With the addition of nine additional types in 1983, coverage was increased to >90% of isolates (*7*). Although the efficacy of this vaccine in various high-risk groups has been debated, the aggregate efficacy in immunocompetent adults is estimated to be ~75% (*8*).

A major problem with the current vaccine is that the purified capsular polysaccharides of pneumococcus do not reliably induce protective antibody responses in children younger than 2 years—the age group that shows the highest incidence of invasive pneumococcal infection and meningitis. In addition, the vaccine appears to confer only limited protection to patients with certain underlying illnesses such as immunodeficiencies and hematologic malignancies, and may not be effective against certain poorly immunogenic capsular types (*8*).

Because polysaccharides are recognized mainly by T cell–independent mechanisms, they do not effectively produce high-level, high-affinity antibodies, particularly in young children, nor do they induce the T cell memory required for booster responses. The immunogenicity of polysaccharides can be increased by covalently linking them to a carrier protein. This approach is thought to work through the recruitment of T cell help (see figure). Hib vaccines developed with this conjugate approach have been enormously successful: They induce high, boostable, and protective antibody levels in infants (*9*), and have dramatically reduced Hib-associated disease in countries where they are in general use. Nevertheless, the serotypic diversity of pneumococcus presents challenging new problems.

The initial strategy of most manufacturers has been to formulate a vaccine containing about seven of the most common pneumococcal types. These include types 4, 6B, 9V, 14, 18C, 19F, and 23F, which together account for more than 80% of invasive isolates from children in the United States (*10*) and most western European countries. In other countries, other serotypes such as types 1, 5, and 7F rank among the most common strains (*11*). From a production vantage, preparation of this vaccine poses formidable challenges, because at least seven separate vaccines must be manufactured, controlled, and combined in appropriate concentrations. Also, in order to achieve 70 to 80% coverage of pneumococcal strains in developing countries, two or three additional strains would need to be added or, less desirably, different formulations would need to be prepared.

From an immunologic vantage, several critical questions must be answered. Can a single protein provide sufficient carrier effect (see figure) for a large number of polysaccharides? Will the large protein load, particularly with repeated injection, lead to severe reactions or to carrier-mediated suppression of the antibody response (*12*)? Will antigenic competition among the serotypes blunt antibody responses, particularly to the weaker polysaccharide antigens? Which carrier protein is most effective? Among those now being studied are a nontoxic cross-reactive variant of diphtheria toxin, tetanus toxoid, diphtheria toxoid, and meningococcal outer membrane protein (OMP). The use of protein carriers already present in the routine diphtheria-tetanus-pertussis (DTP) vaccine may facilitate future combination of the pneumococcal vaccine with DTP, particularly if the conjugates can be prepared by techniques that preserve or even enhance the antibody response to the carrier; this would potentially eliminate the need for carrier protein in both conjugated and free form (*13*). The meningococcal OMP carrier is of particular interest because, unlike tetanus and diphtheria proteins, it induces substantial antibody responses to Hib even after a single dose in 2-month-old infants (*14*). The mechanism of this effect is not fully understood, but may involve direct stimulation by the carrier of T cells and B cells or other direct adjuvant effects (*15*). Potential disadvantages of this carrier are the lower booster responses and the induction of antibodies



Recruiting T cell help for polysaccharide (Ps) antigens. Covalent linkage of polysaccharides to protein antigens is thought to recruit T cell help for Ps-specific B cells by the mechanism shown. B cells with surface immunoglobulin specific for the Ps bind the covalent complex, internalize it, and process the protein antigen into peptides. These peptides (T cell epitopes) are then presented, in the context of class II major histocompatibility complex (MHC) molecules, to T helper (T_H) cells bearing specific T cell receptors (TCR). The activated T_H cells in turn stimulate B cell proliferation and differentiation into antibody-secreting cells. An optimal immune response requires large numbers of T_H cells that recognize the T cell epitopes of the carrier protein. Such T cells are induced by simultaneous or, preferably, prior immunization with the protein-Ps conjugate or with the carrier protein alone. During the first exposure to the carrier protein, expansion of the carrier-specific T cells is most efficient when the protein's T cell epitopes are presented by macrophages or dendritic cells. For this reason, most protein-Ps conjugates induce consistent antibody responses in infants only after two or three doses.

with lower affinity than those induced by other protein carriers (9). However, neither of these factors has been problematic in the case of the Hib-OMP vaccine (9, 16).

Several manufacturers are evaluating pneumococcal conjugate vaccines in phase I and phase II studies in children and infants. Initial results with five- and seven-component vaccines indicate that infants respond well to each serotype, but require three doses to achieve antibody responses reliably. Efficacy trials may begin as early as 1995. A variety of studies are planned, including large-scale trials with sufficient power to detect significant reductions in bacteremic pneumococcal infections (a rare outcome for which high efficacy is expected) and in pneumonia and middle ear infections (common outcomes for which lower rates of efficacy are expected). These trials should also seek to establish serologic correlates of protection so that future vaccines can be evaluated on the basis of antibody responses. To achieve this goal, current efforts to standardize antibody assays by the World Health Organization, the Centers for Disease Control, and the Food and Drug Administration are vital.

Alternative approaches to pneumococcal vaccines are also being considered. Immunization with noncapsular antigens that might induce protection against all serotypes would be advantageous either instead of, or as a complement to, polysaccharide-based vaccines. Such a vaccine could enhance protective efficacy of type-specific conjugate vaccines and extend protection to strains not included in the vaccine. The C-polysaccharide antigen, found in the cell wall of all pneumococci, has been proposed as a candidate (17), as have several protein antigens including pneumolysin, pneumococcal surface protein A (PspA), and a 37-kD surface protein (18). Attractive as the noncapsular antigens are, skeptics point out that the "proof of principle" that they can provide broad-based, species-specific protection has not been satisfactorily demonstrated in animals or in humans (7, 19, 20).

It seems likely that the next generation of pneumococcal vaccines will be based on anticapsular immunity induced by protein-polysaccharide conjugates. Trials may be completed as early as 1998, so these vaccines could come into use before the end of the century. The availability of a vaccine that is effective in infants will enable us, for the first time, to integrate pneumococcal vaccine into routine childhood immunization schedules and thus substantially reduce the burden of pneumococcal disease in the United States and globally. An important remaining challenge will be to reduce the cost of manufacturing protein-polysaccharide conjugate vaccines so that they will be accessible to children throughout the world.

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Cholera Vaccines: Fighting an Ancient Scourge

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Cholera is a potentially lethal diarrheal disease caused by the Gram-negative bacterium *Vibrio cholerae*. This disease has a long history; descriptions of it can be found in Sanskrit, Arabic, and Chinese writings dating back at least two millennia. Since 1871, seven cholera pandemics have occurred (*1*). The first six were probably caused by the classical biotype of *V. cholerae*, whereas the seventh, which began in 1961 and continues today, is caused by the El Tor biotype. Strains of these two biotypes differ in a variety of properties, including production of intestinal adherence factors and other virulence determinants. There have been nearly a million estimated cases of El Tor disease in Latin America since its introduction in Peru in 1991.

Until recently, epidemic strains of both biotypes characteristically produced a serotype lipopolysaccharide O antigen, termed O1, which is known to be one of the primary targets of a protective immune response to cholera. In October 1992, however, a new serogroup of *V. cholerae* (designated O139 or "Bengal") was recognized as the cause of a massive cholera epidemic in South Asia (see figure) (*2*). The O139 strains are closely related to El Tor O1 strains but have acquired new genetic information encoding a distinct lipopolysaccharide O antigen and a polysaccharide capsule (*3*). Epidemiological data suggest that prior immunity to the O1 serogroup of *V. cholerae* offers little protection against the O139 strains. The incidence of cholera in endemic regions of the world, together with the global emergence of new epidemics, emphasize the need for effective cholera vaccines.

Ideally, a cholera vaccine should offer a high degree of safety and should induce long-term immunity against both overt disease and asymptomatic intestinal carriage of *V. cholerae* (the latter being responsible for the majority of infections in endemic areas). The vaccine should also be inexpensive, easy to administer, and afford protection against both the O1 El Tor biotype and the new O139 serogroup. Recent work indicates that the cholera vaccines now under study may approach several of these goals.

Despite 11 decades of research, efforts to develop effective cholera vaccines have produced few successes (*4*). Parenterally administered vaccines (killed whole-cell, lipopolysaccharide, and toxoid) have been largely abandoned because these vaccines induce only weak or short-term immunity. The limited success of these vaccines is attributed to their inability to induce a local intestinal or "mucosal" immune response. Such mucosal immunity appears to be a critical feature of natural convalescence from cholera, a highly immunizing process that provides long-lasting protection.

The strong mucosal immunity elicited by *V. cholerae* results from direct exposure of intestinal epithelial surfaces to bacterial antigens. In principle, orally administered vaccines could provide analogous exposure. Two different types of oral vaccines are being actively pursued. The first is a mixture containing the nontoxic B subunit of cholera toxin and killed whole bacterial

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