Capsular polysaccharide–protein conjugate vaccines

Ramesh K. Sood, Ali Fattom, Viliam Pavliak and Robert B. Naso

The conjugation of polysaccharides to carrier proteins generally enhances polysaccharide immunogenicity and renders the immune response T-cell dependent. Such enhancement of immunogenicity has made the use of conjugate vaccines possible in populations that are otherwise unresponsive to polysaccharide vaccines. Here, the authors discuss the value of capsular polysaccharide vaccines, their ability to elicit protective immunity against infectious bacteria, the selection of appropriate polysaccharides and carrier proteins, and the applications of the resultant conjugate vaccines.

The capsular polysaccharides present on the surface of pathogenic bacteria are virulence factors and protective antigens. Antibodies specific to these polysaccharides have been found to mediate *in vitro* killing of these microorganisms in the presence of complement. Most of these polysaccharides are immunogenic in humans with the exception of children under the age of two, the elderly and adults with compromised immune systems. Unlike protein antigens, polysaccharides are thymus-independent antigens, which generate no anamnestic effect. A second generation of semisynthetic conjugate vaccines, prepared by covalently linking these polysaccharides to carrier proteins, were shown to be efficacious in the immunoprophylaxis of certain invasive bacterial diseases. Polysaccharide–protein conjugates are able to elicit higher amounts of polysaccharide-specific antibodies, and the immune response is T-cell dependent. In this paper we discuss the value of capsular polysaccharide vaccines, and their ability to elicit protective immunity against infectious bacteria. Various factors related to the selection of polysaccharide and carrier protein, which can affect the immunogenicity of the conjugates, are also reviewed. The usefulness of the resultant conjugate vaccines either for active immunization or to generate specific polyclonal immunoglobulins in plasma donors, for use in passive immunization, is also discussed.

Significance of capsular polysaccharides

Most medically important bacteria living in their natural environment are encapsulated by polysaccharides. These capsular polysaccharides (CPs) are virulence-promoting factors. The clearance of capsulated bacteria requires the presence of CP-specific antibodies. Non-encapsulated bacteria, in contrast, are readily cleared by the host. These non-encapsulated organisms can activate host complement in the absence of specific antibodies, resulting in their rapid clearance from the blood. There are several ways by which the presence of the capsule may enhance the virulence of the organism. With a few notable exceptions, such as *Pneumococcus* type 14 and 7F, most CPs are negatively charged at physiological pH. As a result of electronic repulsion, this negative charge on the surface of the bacteria hinders the bacterial cell's required interaction with negatively charged phagocytes during phagocytosis. Moreover, because of its surface location on bacteria, the capsule may interfere with the activation of the complement system by other sub-surface components, resulting in prevention of complement deposition on the bacterial surface or interference with the recognition of these opsonins by the Fc or C3b receptors on the phagocytic cells. In some other cases, for

Ramesh K. Sood*, **Ali Fattom**, **Viliam Pavliak** and **Robert B. Naso**, W.W. Karakawa Microbial Pathogenesis Laboratory, NABI, 12280 Wilkins Avenue, Rockville, MD 20852, USA. *tel: +1 301 770 3099, fax: +1 301 770 2014, e-mail: soodrame%5899452@mcimail.com

  1359-6446/96/$15.00. PII: S1359-6446(96)10032-5

example *Meningococcus* group B, bacteria may evade the host immune system by expressing capsules containing sialic acid, thereby making it difficult for the immune system to recognize them as foreign antigens. Thus, CPs contribute significantly to the virulence of some pathogenic bacteria by enhancing their survival in the blood, because they enable bacteria to evade the host's defense mechanisms.

Capsular polysaccharides as vaccines

The discovery of bacterial CPs dates back to the identification of specific soluble substances[1] secreted by pneumococci. These substances were later identified as carbohydrates by Heidelberger and Avery[2]. The importance of these substances in immunological protection of the host was demonstrated by the protective effect of pneumococcal polysaccharide-specific antibodies against pneumococcal infections[3,4]. CPs make up a group of polymers with almost unlimited structural variation. They consist of small units ranging from one to several sugar residues in length that are repeated to make up a polymeric chain. Most of the CPs show pronounced immunological specificity, generally determined by relatively small portions of the polysaccharide, called antigenic determinants, which are usually specific for a given serotype. However, the presence of the same determinant group in polysaccharides from nonrelated species is responsible for serological cross-reactivity.

The use of polysaccharides as antigens and immunogens has contributed greatly to the classification and identification of bacteria, as well as to the detection and prevention of human disease caused by invasive microorganisms. The potential of CPs as vaccines was first fully confirmed by the demonstration that multivalent pneumococcal polysaccharide vaccines were able to provide type-specific protection in humans against pneumococcal pneumonia[3]. At that time, however, the phenomenal success of antibiotics in the treatment of bacterial infections rapidly restricted the further development of polysaccharide based vaccines. Later findings that antibiotic treatment of infectious diseases caused by encapsulated bacteria does not always prevent their morbidity and mortality[5], and the frequent incidence of antibiotic-resistant strains resulted in renewed interest in the immunoprophylaxis of bacterial diseases.

At the moment, several CP vaccines are licensed for use in the immunoprophylaxis of pneumococcal pneumonia and meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Ref. 6). CPs are, with few exceptions, immunogenic in humans, nontoxic and free of other deleterious effects associated with vaccines derived from whole killed bacteria. Unfortunately, a number of problems restricting further development of polysaccharides as vaccines have been identified, the most important being the poor immunogenicity of some CPs, particularly in children below the age of two, the elderly and immunocompromised patients[7–10]. Unfortunately, these same populations are those at greatest risk of infection. For most immunogens, including polysaccharides attached to the bacteria, the production of antibody is based on the cooperative interaction of two types of lymphocytes, T cells (thymus derived) and B cells (bone marrow derived). This antibody response can be boosted to higher levels on further exposure to the antigen (anamnestic response) with the production of antibodies of various IgG isotypes. However, pure polysaccharides (removed from the bacteria) are considered to be T-cell-independent antigens, because they are capable of inducing only immune responses with no anamnestic effect. The immunogenicity of polysaccharides appears to be linked closely to the size of the molecule in normal adults. Antibody levels induced by immunogenic polysaccharides are high enough to provide effective protection against bacterial infection and remain at this high level for months or even years[11]. On the other hand, infants respond very poorly to polysaccharide vaccines[5,7,12–14]. The immune response of children to different polysaccharides is age-related and also depends on polysaccharide structure[15]. The maturation process of the immune response of infants to polysaccharides occurs during the first few years of life[7], after which a child is as capable of responding to a polysaccharide vaccine as is an adult.

Capsular polysaccharide–protein conjugate vaccines

To overcome the restricted immunogenicity of polysaccharide vaccines, a new generation of semisynthetic vaccines based on the conjugation (covalent coupling) of polysaccharides to protein carriers have been explored in recent years. The first attempts to potentiate the immune response to polysaccharides by linking them covalently to a protein were made by Avery and Goebel[16,17]. Six decades later, in 1987, the first conjugate vaccine was approved for the prevention of invasive diseases caused by *H. influenzae* type b for children aged 18 months to six years, and in 1990 its use was extended to include infants[18]. In contrast to the restricted and otherwise poor immunogenicity of polysaccharides, polysaccharide–protein conjugates were shown to

elicit high levels of polysaccharide-specific antibodies. Conjugates were also shown to induce a boostable antibody response. These conjugate vaccines were able to elicit a high level of polysaccharide-specific antibodies in infants, which protected them from *H. influenzae* type b infections[19,20] during the early years in life when they are at high risk of contracting infection by these bacteria. The phenomenal success of the currently licensed CP–protein conjugate vaccines against the invasive diseases caused by *H. influenzae* type b in infants has led to the development and clinical evaluation of several other conjugate vaccines against various other bacterial pathogens. Development of these vaccines has involved the use of different conjugation technologies and carrier proteins.

Design of conjugate vaccines

The design of conjugate vaccines has two essential elements: selection of the appropriate individual components, i.e. the saccharide and the protein; and selection of an appropriate method to link these two essential components through a covalent bond. The chemistry used to form these chemical linkages between polysaccharide and protein will depend on the nature of the available functional groups on the individual components, and the stability of certain labile functional groups present on the saccharide or protein under the reaction conditions employed for conjugation.

Selection of the saccharide

Bacterial polysaccharides, isolated in their native form, exist in a wide range of molecular sizes and, unlike proteins, even the polysaccharide isolated from the same bacterial culture is heterogeneous in terms of molecular weight distribution. The foremost question in the design of conjugate vaccines is whether there is an optimal size of saccharide for preparing these vaccines. The published literature does not point towards a simple answer to this question. Conjugates prepared from small oligosaccharides from dextrans[21] and *Salmonella typhimurium*[22] O-antigen type 4 were found to be more immunogenic in animals than conjugates prepared from larger polysaccharides. In contrast, conjugates from the high molecular weight polysaccharides in Vi antigen[23] and *Pneumococcal* type 4 (Ref. 24) were found to be more immunogenic than the conjugates prepared from smaller oligosaccharides. In group B *Streptococcus* type III, antibodies elicited in response to intermediate-size polysaccharide conjugates provided superior protection in mice against challenge by live bacteria[25].

From these studies, it appears that the optimal saccharide size may depend on the nature of the polysaccharide and should be defined individually for each specific polysaccharide. During the development of a conjugate vaccine, it is advisable to do a systematic study to find the optimal length of the polysaccharide. However, the evaluation of such prepared conjugates in animals may not be sufficient to decide the optimal polysaccharide size for use in humans. Anderson[26] showed that the conjugate prepared from the 20-repeating-unit polysaccharide of *H. influenzae* type b proved superior in the priming of an infant's immune response to the saccharide component, compared with conjugate prepared from eight repeating units of polysaccharide. However, this relationship could not be established in the animal studies, where both conjugates were equally immunogenic.

Selection of carrier protein

For a protein to be an acceptable carrier in a conjugate vaccine, it must be nontoxic and it must have epitopes suitable for its interaction with T cells. These criteria provide numerous candidates to choose from. In practice, however, only a handful of proteins of bacterial origin have been used for the preparation of the conjugate vaccines that have been licensed for human use or are currently under development. The most commonly used carrier proteins, like tetanus toxoid (TTd) and diphtheria toxoid (DTd), are constituents of existing DPT vaccines. Such proteins can provide protection against toxins produced by corresponding bacteria, provided that the T-cell and B-cell epitopes of these proteins are conserved during the conjugation process. These toxoids are prepared from the native toxins by treating the toxins with formaldehyde to destroy some of the readily accessible amino groups on lysine residues; this process is called toxoiding. Excess toxoiding can result in a carrier protein that is unable to induce antibodies that recognize native toxin. This may be a disadvantage if the vaccine is intended for protection against diseases caused by toxin production. Toxoiding may also have the down-side of limiting the number of available lysine amino groups for use during any subsequent conjugation process. Although this has not been proven to be a major problem, it may still be desirable to have the maximum number of available sites for conjugation, in order to achieve more efficient conjugation.

The problem caused by toxoiding may be avoided by using recombinant-DNA-derived toxin mutants, which do not require toxoiding. Such mutants generate nontoxic

carrier proteins like CRM_{197} (the antigenic equivalent of diphtheria toxin), which are immunologically cross-reactive with the toxin[26–28]. Another example of the use of a non-toxic, toxin-derived recombinant carrier protein is exoprotein A (rEPA), derived from *Pseudomonas aeruginosa* exotoxin A by deletion of an amino acid at the active site of the toxin[29,30]. The problem of limited availability of free amino groups on protein toxoids for use in conjugation may also be avoided by the use of the carboxyl groups from the acidic amino acid residues of the carrier proteins for conjugation[31].

Another category of proteins of bacterial origin that have been used for conjugate preparations are the outer membrane proteins (OMP) from pathogenic bacteria like *N. meningitidis*[32] and *H. influenzae* type b[33]. A major problem associated with the use of these proteins is the difficulty in purifying OMP from the bacterial source. Proteins purified from Gram-negative bacteria may be contaminated with toxic lipopolysaccharides, which can cause toxicity. One possible solution to this problem is to obtain these proteins by recombinant technology[34,35].

Saccharide–protein conjugation

Once the polysaccharide and the carrier protein to be used for conjugation have been selected, the covalent linking of the two entities can be achieved in various ways. Oligosaccharides and relatively small polysaccharides can be linked to proteins through either their reducing end group only[27,36,37], or through their terminal ends[26,38] by reductive amination following generation of aldehyde groups on either end. For the large polysaccharides, end group linkage becomes inefficient and, moreover, the resulting conjugates exhibit more undesired T-cell-independent properties than the desired T-cell-dependent properties[39]. In these cases, certain functional groups along the polysaccharide chain can be activated in a random fashion, and then used for conjugation to proteins either directly or indirectly through the use of linker arms. Conjugates made with both terminally activated[26] and randomly activated[31,32,40,41] *H. influenzae* type b polysaccharides have been approved as vaccines for infants. Although this would indicate that there is no functional advantage of one method over the other, the random activation may provide some other practical advantages. The use of native polysaccharide without depolymerization, in addition to being convenient from the manufacturing perspective, also minimizes material loss that may be incurred during depolymerization steps. Moreover, the depolymerization step requires the development of methods to depolymerize these polysaccharides to a consistent size. The final structure of the conjugate produced by any method depends greatly on the chemistry used during conjugation. Conjugates produced from the single-end-group-linkage methods have simple structures with saccharide chains radiating from the main protein backbone without any cross-linking. Conjugation methods that generate random linkages along the chain, however, lead to a conjugate with a highly cross-linked lattice structure[42].

The prime consideration during the conjugation process is to achieve a covalent linkage between the polysaccharide and the carrier protein without bringing about any significant changes to the structures of the individual components. Bacterial polysaccharides contain a wide array of acid- or base-sensitive functional groups like *O*-acyl groups[43], phosphate groups[44] and sialic acid residues[45]. These functional groups may constitute an important part of the immunodominant epitopes. Since the immunogenicity of the given saccharide or protein may be a function of its three-dimensional structure, the methods employed for the conjugation must be mild enough to maintain the integrity of these functional groups and retain the three-dimensional conformation of the individual components. CPs are polyfunctional molecules containing neutral functional groups like hydroxyl, carbonyl and amino groups, or charged groups like carboxyl or phosphate residues. Sometimes new functional groups can be introduced into the molecule by chemical manipulation of the existing groups. The most common functional groups on proteins, which are used during conjugation, are the side-chain amino groups of lysine residues or the carboxyl groups on the side-chains of acidic amino acid residues. In theory, any of the above groups can be used for conjugation; however, selective activation and steric accessibility of these groups during conjugation, coupled with the stability of the resulting covalent linkage, may limit the choice.

Polysaccharides can be activated by different methods prior to their conjugation to carrier proteins. The conjugation methodology and the choice of carrier protein seem to have little effect on the immune response to the resulting conjugates in animals[30]. For example, when conjugates of *S. aureus* type 8 CP with rEPA or DTd, prepared using different conjugation methodologies, were evaluated in mice, the immune responses to these conjugates were comparable and the conjugates exhibited the general properties of T-cell-dependent response, regardless of the conjugation

methodology or the carrier protein used. Antibodies elicited by these conjugates were evaluated for their IgG-subclasses distribution. Most (80–90%) of the IgG elicited by these conjugates was of the IgG1 subclass and the remaining 10% was composed of the other subclasses[46]. These data suggest that the major requirement for imparting T-cell-dependent properties to a polysaccharide is its conjugation to carrier protein. A factor that does play a major role in defining the optimum immunogenicity of the conjugate vaccine is the polysaccharide-to-protein ratio in the conjugate. There is an optimum range for this ratio and the presence of too much or too little of either component in the final conjugate can be detrimental to the immunological response. The optimal ratio of polysaccharide to protein is likely to be polysaccharide-, carrier protein- and conjugate-specific and must be determined experimentally.

Major advances in the glycoconjugate-chemistry field in the last 15 or 20 years have resulted in the development of several comparatively 'mild' methods for saccharide–protein conjugation. These methods provide flexibility in selecting the functional groups to be used during conjugation, in selecting conditions to maintain the integrity of acid- or base-labile subgroups, and in the choice of carrying out conjugation with or without the introduction of small foreign molecules to link the polysaccharide to the protein; these small molecules are called linkers. The final choice of method in every case will depend on the size and structure of the given saccharide. A detailed description of these methods is beyond the scope of this review and the readers are referred to earlier reviews that provide detailed descriptions of these methods[42,47–49].

Immunogenicity of polysaccharide–protein conjugates

As previously described, conjugation of polysaccharide to protein converts the former from a T-cell-independent to a T-cell-dependent antigen, which elicits a higher and boostable immune response in animals[50]. Priming of animals with one injection of carrier protein followed by one injection of conjugate elicits an immune response equivalent to two injections of conjugate[29,51,52]. These results are characteristic of T-cell-dependent antigens: they elicit a booster response as shown by carrier priming. This transformation from T-cell-independent to T-cell-dependent antigen requires the saccharides to be covalently bound to the carrier protein. Combining the two antigens by mixing, or even affinity association (for example avidin–biotin complexes), usually fails to enhance the immunogenicity of the saccharide or to confer T-cell-dependent properties on the saccharide (D. Watson, pers. commun.).

Combination conjugate vaccines and interference

Bacteria that cause disease in humans usually possess a variety of serologically distinct capsular polysaccharides. The pneumococcal vaccine was initially composed of 14 of the most prevalent pneumococcal capsular polysaccharides; this vaccine was later replaced by a 23-valent vaccine[53,54]. The combination of multiple polysaccharides into a single formulation did not appear to cause any problem with regard to the performance of any individual component in the mixed formulation, when compared with the performance of components administered individually; this is apparently due to the fact that the immune response to the unconjugated polysaccharides is T-cell-independent and mediated through B cells only[55]. Conjugation of CP to carrier protein, however, means that the carrier protein is the component that is recognized by specific T cells, which now participate in the immune response to the polysaccharide. Having too many saccharide antigens dependent on a single carrier protein in the same injection, appears, at times, to induce competition among the different components for a limited T-cell population capable of recognizing the carrier protein; thus the immune response to one or more saccharide components may be reduced.

It was recently shown that some components of the polyvalent *Pneumococcus* polysaccharide vaccine were more immunogenic than corresponding components of a polyvalent conjugate vaccine[56]. We also observed such antigenic interference in animals when *S. aureus* type 5 and type 8 conjugates were combined into one injection[57]. Similar interference was observed when the *H. influenzae* type b conjugate was combined with the DPT vaccine[58–60]. In this latter case, the reduced immune response to the polysaccharides was attributed to the presence of pre-existing carrier-specific antibodies. However, in certain cases the presence of carrier-specific pre-existing antibodies has been shown to enhance the immune response to a polysaccharide[58]. It is theorized that since T cells recognizing a specific carrier protein are limited in number, each conjugate component will compete for T cells in the population. This competition may well reduce the number of T cells that will be presented with any specific saccharide type in a mixture, thus reducing the T-cell help conferred on that antigen. The insufficiency of the carrier–saccharide primed T-cell

population presumably results in a reduced immune response to individual components of a saccharide mix in comparison with the response to the specific saccharide-conjugate when used individually. Experiments are now ongoing to test this theory. This problem of antigenic interference must be addressed if multiple conjugates are to be administered in combined vaccines.

Active and passive immunization

Two other issues affecting the future development of polysaccharide vaccines and their use are the time required to mount an effective immune response to the vaccine and the immunocompetence of the proposed recipient population. Populations at higher risk of infections may be unable to mount an immune response that will be adequate to combat infections in a timely manner. Low-birthweight neonates, for example, are at risk from bacterial infections, but are not immunocompetent. Trauma patients are admitted to the hospital following an injury and are at immediate risk from hospital-acquired infections; while they may be immunocompetent, they do not have time to mount an immune response to a vaccine.

In one study at the shock-trauma and intensive-care units of the University of Maryland Hospital in Baltimore, all *S. aureus* bacteraemia and pneumonia infections in admitted patients were found to occur between day 2 and day 15 of hospitalization[61,62]. Obviously, for these and certain other at-risk populations, active immunization is impractical. One rational and practical approach to providing such individuals with immunity is to passively immunize these patients with specific hyperimmune gammaglobulins. Such gammaglobulin preparations may be produced from the plasma of healthy plasma donors, who have been immunized with the appropriate vaccine. To this end, we produced intravenous immunoglobulin (IGIV) from the plasma of donors immunized with an experimental bivalent *S. aureus* type 5 and type 8 CP–rEPA conjugate (StaphVAX™, NABI). In comparison with the commercially available standard IGIV, the specific hyperimmune IGIV (StaphGAM™, NABI) contained 30–40 times more *S. aureus* type 5 and 8 CP-specific antibody. This preparation of StaphGAM™ was found to be opsonic in an *in vitro* opsonophagocytic assay and was protective in animal model studies[63] following challenge with *S. aureus*. This vaccine-induced antibody product may have an advantage over either screened or standard IGIV by having a higher specific immunoglobulin content. Moreover, preliminary results have shown that the vaccine-induced immunoglobulin had greater than fourfold higher affinity for *S. aureus* CP than did commercial standard IGIV[64]. Studies are ongoing to optimize the donor-vaccination regimen, the dose of vaccine for donor stimulation, and to evaluate the effect of plasmapheresis frequency on the specific-antibody levels in plasma donors.

Summary

The conjugation of polysaccharides to carrier proteins generally enhances polysaccharide immunogenicity and renders the immune response T-cell dependent. This enhancement of polysaccharide immunogenicity has made possible the use of conjugate vaccines in populations, such as young children and immunocompromised patients, which are otherwise unresponsive to polysaccharide vaccines. Effective vaccines composed of capsular polysaccharides have been developed and introduced into the marketplace; this has led to the development and clinical evaluation of several additional conjugate vaccines against various bacterial pathogens. The selection of polysaccharide, carrier protein and conjugation technology may depend upon a number of factors, and a conjugate with optimal immunogenicity may, in turn, be dependent upon the individual components selected. The combination of multiple polysaccharide conjugates into multicomponent vaccines may be complicated by antigen interference, which may be overcome by use of different carrier proteins. Since conjugates generally elicit higher antibody titers than saccharides alone, conjugate vaccines may also be most appropriate for use in immunization of plasma donors for the production of antibodies for passive immunization. Such antibodies may provide an alternative to active vaccination for the prevention and treatment of bacterial infections.

REFERENCES

1 Dochez, A.R. and Avery, O.T. (1917) *J. Exp. Med.* 26, 477–493
2 Heidelberger, M. and Avery, O.T. (1923) *J. Exp. Med.* 38, 73–79
3 MacLeod, C.M. *et al.* (1945) *J. Exp. Med.* 82, 445–465
4 Heidelberger, M. *et al.* (1946) *J. Exp. Med.* 83, 301–320
5 Robbins, J.B. (1978) *Immunochemistry* 15, 839–854
6 Jennings, H.J. (1990) *Curr. Top. Microbiol. Immunol.* 150, 97–127
7 Gotschlich, E.C., Goldschneider, I., Lepow, M.L. and Gold, R. (1977) in *Antibodies in Human Diagnosis and Therapy* (Haber, E. and Krause, R.M., eds), pp. 391–402, Raven
8 Winston, D.J. *et al.* (1983) *Arch. Intern. Med.* 143, 1735–1737
9 Bolan, G. *et al.* (1986) *Ann. Intern. Med.* 104, 1–6
10 Reingold, A.L. *et al.* (1985) *Lancet* ii, 114–118
11 Heidelberger, M. *et al.* (1950) *J. Immunol.* 65, 535–541
12 Peltola, H. *et al.* (1977) *Pediatrics* 60, 730–737
13 Austrian, R. (1985) *Ann. Inst. Pasteur Microbiol.* 136B, 295–307

14 Robbins, J.B., Parke, J.C., Schneerson, R. and Whisnant, J.K. (1973) *Pediatr. Res.* 7, 103–110
15 Douglas, R.M., Paton, J.C., Duncan, S.J. and Hansman, D.J. (1983) *J. Infect. Dis.* 148, 131–137
16 Avery, O.T. and Goebel, W.F. (1929) *J. Exp. Med.* 50, 521–533
17 Goebel, W.F. (1939) *J. Exp. Med.* 69, 353–364
18 Stein, K.E. (1994) *Int. J. Technol. Assess. Health Care* 10, 167–176
19 Santosham, M. *et al.* (1991) *New Engl. J. Med.* 324, 1767–1772
20 Black, S.B. *et al.* (1991) *Pediatr. Infect. Dis. J.* 10, 97–104
21 Seppala, I. and Makela, O. (1989) *J. Immunol.* 143, 1259–1264
22 Jorbeck, H.H.A., Svenson, S.B. and Lindberg, A.A. (1981) *Infect. Immun.* 32, 497–502
23 Szu. S. *et al.* (1989) *Infect. Immun.* 57, 3823–3827
24 Peeters, C.C.A.M. *et al.* (1991) *J. Immunol.* 146, 4308–4316
25 Paoletti, L.C. *et al.* (1992) *J. Clin. Invest.* 89, 203–209
26 Anderson, P.W. *et al.* (1986) *J. Immunol.* 137, 1181–1186
27 Anderson, P. (1983) *Infect. Immun.* 39, 233–238
28 Anderson, P., Pichichero, M.E. and Insel, R.A. (1985) *J. Clin. Invest.* 76, 52–59
29 Fattom, A. *et al.* (1990) *Infect. Immun.* 58, 2367–2374
30 Fattom, A. *et al.* (1992) *Infect. Immun.* 60, 584–589
31 Chu, C. *et al.* (1983) *Infect. Immun.* 40, 245–256
32 Marburg, S. *et al.* (1986) *J. Am. Chem. Soc.* 108, 5282–5287
33 Evenberg, D. *et al.* (1992) *J. Infect. Dis.* 165 (Suppl. 1), S152–S155
34 Paton, J. *et al.* (1991) *Infect. Immun.* 59, 2297–2304
35 Lucak, M., Pier, G.B. and Collier, R.J. (1988) *Infect. Immun.* 56, 3095–3098
36 Jennings, H.J. and Lugowski, C. (1981) *J. Immunol.* 127, 1011–1018
37 Porro, M. *et al.* (1985) *Mol. Immunol.* 22, 907–919
38 Beuvery, E.C., Roy, R., Kanhai, V. and Jennings, H.J. (1986) *Dev. Biol. Stand.* 65, 197–204
39 Jennings, H.J. (1992) *J. Infect. Dis.* 165 (Suppl. 1), S156–S159
40 Gordon, L.K. (1986) US Patent 4,619,828
41 Marburg, S., Tolman, R.L. and Kniskern, P.J. (1989) US Patent 4,882,317
42 Dick, W.E., Jr and Beurret, M. (1989) in *Contributions to Microbiology and Immunology* (Vol. 10) (Cruse, J.M. and Lewis, R.E., Jr, eds), pp. 48–114, S. Karger
43 Moreau, M. *et al.* (1990) *Carbohydr. Res.* 201, 285–297
44 Bundle, D.R., Smith, I.C.P. and Jennings, H.J. (1974) *J. Biol. Chem.* 249, 2275–2281
45 Wessels, M.R. *et al.* (1987) *J. Biol. Chem.* 262, 8262–8267
46 Fattom, A. *et al.* (1995) *Vaccine* 13, 1288–1293
47 Jennings, H.J. and Sood, R.K. (1994) in *Neoglycoconjugates: Preparation and Applications* (Lee, Y.C. and Lee, R.T., eds), pp. 325–371, Academic Press
48 Aplin, J.D. and Wriston, J.C., Jr (1981) *CRC Crit. Rev. Biochem.* 10, 259–306
49 Stowell, C.P. and Lee, Y.C. (1980) *Adv. Carbohydr. Chem. Biochem.* 37, 225–281
50 Robbins, J.B. and Schneerson, R. (1990) *J. Infect. Dis.* 161, 821–832
51 Schneerson, R. *et al.* (1986) *Infect. Immun.* 52, 519–528
52 Schneerson, R. *et al.* (1980) *J. Exp. Med.* 152, 361–376
53 Austrian, R. (1981) *Rev. Infect. Dis.* 3, S51–S57
54 Robbins, J.B. *et al.* (1983) *J. Infect. Dis.* 148, 1136–1159
55 Golding, B. (1991) in *Topics in Vaccine Adjuvant Research* (Spriggs, D.R. and Koff, W.C., eds), pp. 25–37, CRC Press
56 Molrine, D.C. *et al.* (1995) *Ann. Intern. Med.* 123, 828–834
57 Cho, Y.H., Fries, L., Naso, R. and Fattom, A. (1995) *35th ICAAC*, San Francisco, p. 172 (Abstr.)
58 Peeters, C.C.A. *et al.* (1991) *Infect. Immun.* 59, 3504–3510
59 Barington, T. *et al.* (1993) *Infect. Immun.* 61, 432–438
60 Barington, T. *et al.* (1994) *Infect. Immun.* 62, 9–14
61 Fattom, A. *et al.* (1993) *33rd ICAAC*, New Orleans, p. 331 (Abstr.)
62 Fattom, A. *et al.* (1995) *95th American Society of Microbiology General Meeting*, Washington DC, p. 275 (Abstr.)
63 Fattom, A. *et al. Infect. Immun.* (in press)
64 Basham, L. *et al.* (1996) *96th American Society of Microbiology General Meeting*, New Orleans, p. 534 (Abstr.)

In short ...

GlaxoWellcome and **SmithKline Beecham** have announced an agreement to collaborate on the complete genomic sequencing of a number of disease-causing microorganisms. They will then use the data obtained in their own R&D programmes, operating independently and in open competition.

The first phase of the integration process of **Ciba** and **Sandoz** to form **Novartis** is nearing completion. There have been 3,500 new management designations worldwide recently. Novartis expects to fill all further management vacancies by the end of the year.

Amgen has licensed **Yamanouchi Pharmaceuticals** to develop, manufacture and market its Consensus Interferon (CIFN), a synthetic interferon indicated for the treatment of hepatitis C. The license does not include the marketing rights for the USA and Canada, where Amgen will market CIFN as INFERGEN®.

Amgen also announced that its Senior Vice President and Head of Research, Daniel Vapnek, is retiring after 15 years with the company.

Zeneca reports that the FDA has cleared its broad-spectrum intravenous antibiotic Merrem for use in treating serious bacterial infections, such as complicated intra-abdominal infections and bacterial meningitis. It is the first carbapenem antibiotic available in the USA that can be used to treat children.

Heliosynthese and **Scotia Pharmaceuticals** have received an EEC Eureka award for their joint microalgae programme, known as 'Alga–Omega'. The programme is designed to identify sources of polyunsaturated lipids, particularly arachidonic acid and docosahexanoic acid, in microalgae. These lipids are expected to find wide application as nutritional and pharmaceutical products.