

Review article

Novel vaccine strategies to T-independent antigens

Gregory B. Lesinski^a, M.A. Julie Westerink^{a,b,*}

^a Department of Pathology, Medical College of Ohio, Toledo, OH, USA

^b Department of Medicine, Medical College of Ohio, Toledo, OH, USA

Received 25 January 2001; received in revised form 2 April 2001; accepted 17 May 2001

Abstract

T cell independent antigens do not require T cell help to induce an immune response, and are characterized by a lack of immunologic memory. These antigens can be divided into two classes, TI-1 or TI-2. TI-1 antigens, such as bacterial lipopolysaccharide, are potent B-cell mitogens, capable of non-specific, polyclonal activation of B cells. In contrast, TI-2 antigens can only activate mature B cells and consist of highly repetitive structures, such as capsular polysaccharides (CPS) from bacteria. Many vaccines currently in use consist of purified capsular polysaccharides from pathogenic bacteria such as *Streptococcus pneumoniae* and *Neisseria meningitidis*. These vaccines are efficacious in immune-competent adults, however, due to their TI-2 nature, are not effective in children < 2 years of age. Converting polysaccharides into T cell dependent (TD) antigens, allows children, < 2, to produce an effective immune response. This review focuses on various strategies used to convert the immune response to polysaccharide antigens from TI-2 to a TD response. Conjugate vaccines, anti-idiotypic antibodies, phage display library technology and DNA vaccines are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Anti-idiotypic antibodies; DNA vaccines; Peptide mimicry; *Neisseria meningitidis*; *Streptococcus pneumoniae*; TI-2 antigens

1. Introduction

A central tenet of immunology today is that an antigen can be classified as T cell dependent (TD) or T cell independent (TI) in nature. TD antigens are proteins or peptides that require immune stimulation from helper T cells to elicit an immune response. Such antigens are presented to T cells in the context of MHC molecules on macrophages, B cells or

dendritic cells following bacterial or viral infection. The subsequent activation of T cells induces cytokine production and an array of immunologic effects. TD antigens are effective at inducing a lasting immune response, forming memory B and T cells, and producing high affinity antibodies of multiple isotypes.

In contrast, TI antigens do not produce memory or require T cell help to elicit an immune response. These antigens are generally polysaccharides and as a result, cannot be presented to T cells via MHC molecules. TI antigens are further separated into two individual classes, TI-1 or TI-2 based on their interaction with B cells. TI-1 antigens, such as lipopolysaccharide (LPS) are known as potent B cell mito-

* Corresponding author. Department of Medicine, Medical College of Ohio, PO Box 10008, Toledo, OH 43699-0008, USA. Tel.: +1-419-383-4897; fax: +1-419-383-3075.

E-mail address: mwesterink@mco.edu (M.A.J. Westerink).

gens, which function by non-specific, polyclonal activation of most B cells. Because all signals for B cell activation originate from the antigen itself, TI-1 antigens do not induce memory, isotype switching of antibodies or T-helper cell involvement. TI-2 antigens consist of highly repetitive structures such as capsular polysaccharides from bacteria. Unlike TI-1 antigens, these antigens do not function as B cell mitogens, and can only activate mature B cells. It is generally accepted that TI-2 antigens activate B cells by cross-linking surface exposed immunoglobulin. As a result, the activated B cell will produce antigen-specific antibodies. The mechanism for immune responses to TI-2 antigens is not completely understood. For example, various studies have suggested a role of $\gamma\delta$ -T cells (Williams, 1998) and CD5 B cells (Neron and Lemieux, 1997) in the TI-2 immune response. Despite questions regarding the exact mechanism, it has been known for years that certain populations do not respond well to TI-2 antigens. Examples of TI-2 non-responders include small population subsets, such as asplenic patients and HIV seropositive patients. More importantly, children (< 2 years of age) and the elderly (adults > 65 years of age) often do not respond well to TI-2 antigens. The inability of such populations to effectively respond to TI-2 antigens continues to be a challenge in vaccine development against encapsulated pathogens.

2. Limitations of polysaccharide vaccines

Multi-serotype polysaccharide-based vaccines have been in use for both *Streptococcus pneumoniae* and *Neisseria meningitidis* for over 15 years. These vaccines are based on the observation that antibodies against capsular polysaccharide protect against disease by inducing complement-mediated bactericidal antibody (Goldschneider et al., 1969; Gotschlich et al., 1969) and opsonophagocytosis (MacLeod et al., 1945). Each year, in the United States alone, *S. pneumoniae* is responsible for 500,000 cases of pneumonia, 50,000 cases of sepsis, 3000 cases of meningitis and finally 40,000 deaths (Poland, 1999). In underdeveloped countries, the disease burden is even more evident as 1.2 million children, < 2 years

of age, die from pneumococcal disease each year. The presently available pneumococcal vaccine contains capsular polysaccharides from 23 of the over 90 pneumococcal serotypes. This vaccine covers approximately 90% of invasive pneumococcal isolates (Robbins et al., 1983). The vaccine efficacy in immune-competent adults ranges from 56% to 75%. However, due to the TI-2 nature of the immune response to carbohydrate (COOH) antigens, children, < 2 years of age, do not respond to this vaccine.

N. meningitidis is a major human pathogen and continues to be one of the leading causes of bacterial meningitis throughout the developed and developing world. Incidence of meningococcal disease is endemic in the United States, yielding 1.1 cases/100,000 or an estimated 2400 cases per year (Rosenstein et al., 1999). In sub-Saharan Africa, however, epidemic meningococcal disease continues to be a major health problem. Attack rates generally range from 100 to 500/100,000 with an incidence of up to 10% reported in affected villages (Greenwood et al., 1987). Periodic outbreaks are also increasing in frequency in both the United States and developing (Denamur et al., 1987; Pinner et al., 1992; Jackson et al., 1995; Whalen et al., 1995a). The current meningococcal vaccine is similar to the pneumococcal construct. This vaccine contains capsular polysaccharides from a limited number of meningococcal serogroups including A, C, Y and W135. The quadrivalent meningococcal polysaccharide vaccine is not routinely administered but has been recommended for the control of meningococcal serogroup C outbreaks (Anonymous, 2000). Several controlled field trials have been performed in adults and estimate the vaccine efficacy of the serogroup C meningococcal vaccine between 86% and 91% (Artenstein et al., 1970; Gold and Artenstein, 1971; Biselli et al., 1993). The TI-2 nature of this vaccine limits its utility as a worldwide strategy for meningococcal disease prevention. In addition, it does not protect against meningococcal serogroups not included in the vaccine, namely serogroup B. This serogroup alone is responsible for up to 80% of meningococcal infections in European countries (Jones, 1995) and approximately 30% of meningococcal disease in the United States (Rosenstein et al., 1999). Because of the high cost, the dependence on refrigeration for transport and the T-independent na-

ture of the immune response, researchers continue to investigate alternative vaccine strategies against the pneumococcus and meningococcus.

Since polysaccharide-directed antibodies offer protective immunity, capsular polysaccharides are attractive vaccine candidates. To overcome the problems associated with the TI nature of PS antigens, vaccine strategies against these organisms have focused on converting the TI-2 immune response to that of a TD immune response. Successful conversion to TD immunity would allow high affinity antibody production and memory B cell formation against carbohydrate antigens. This strategy could be used against many TI-2 antigens on pathogens, or ultimately on tumor cells. The present review will focus on strategies to convert TI-2 antigens to TD antigens. These include conjugate vaccines that continue to use the polysaccharide as an antigenic component. In contrast, other strategies, namely anti-idiotypic antibodies, bacteriophage display libraries and genetic immunization or DNA vaccines utilize the concept of peptide mimics of polysaccharide antigen. Applying these strategies to developing vaccines against the meningococcus and the pneumococcus will be discussed.

3. Conjugate vaccines

Conjugate vaccines consist of purified capsular polysaccharide or oligosaccharide antigens covalently linked to immunogenic carrier proteins. This formulation converts the polysaccharide to a TD antigen and increases its immunogenicity. It is hypothesized that PS-specific B cells internalize the PS-carrier complex. Proteolysis of the carrier protein produces peptides that bind to class II MHC molecules and activate helper T cells (McCool et al., 1999). As a result, PS-specific B cells can then mature to antibody producing plasma cells or into memory cells (Schneerson et al., 1980; Biselli et al., 1993; Siber, 1994). Such a vaccine strategy was first developed by Avery (1929) by preparing a protein conjugate of polysaccharide from *S. pneumoniae* serotype 3. This polysaccharide, normally a weak immunogen, produced a protective antibody response when conjugated to a carrier protein. This break-

through was the first example of memory cell formation to a polysaccharide antigen. Despite these findings, efforts to develop other conjugate vaccines may have been overshadowed by the introduction of antibiotics. As a result, therapy rather than prevention became the precedent for responding to bacterial infection (Schneerson et al., 1980).

In the mid 1900s, reports of antibiotic resistant microorganisms raised concern that improved vaccines were needed to complement appropriate antimicrobial therapy. In addition, improved vaccine design resulted from advances in understanding the role of B and T cells, polysaccharide structure and mechanisms of immunologic memory (Lindberg, 1999). The first conjugate vaccine introduced for use in humans was against *Haemophilus influenzae* type B (Hib) (Granoff et al., 1993). A polysaccharide-based Hib vaccine in use at the time, however, conferred no protection to children < 2 years due to its TI-2 nature (Holmes et al., 1991). Since this population was at highest risk for Hib disease, vaccine development focused on conversion to a TD immune response. In December of 1987, the first Hib conjugate vaccine was licensed for use in the United States. By the end of 1990, four Hib conjugate vaccine formulations were available and recommended for infants beginning at 2 months of age (Holmes et al., 1991). A dramatic decline of Hib disease occurred in areas where the Hib conjugate was introduced. It was estimated that a $\geq 97\%$ reduction in disease occurred in Finland, the UK and the USA (Lindberg, 1999). Such a decrease in incidence is likely due to the ability of the Hib conjugate vaccines to reduce colonization with Hib bacteria in the nasopharynx. This suggests a protective activity exerted at mucosal surfaces leading to decreased disease in non-immunized individuals, due to decreased exposure (Takala et al., 1991).

The success of the Hib conjugate vaccine has prompted the development of a conjugate vaccine against *S. pneumoniae*. Like Hib-PS, antibodies against pneumococcal polysaccharide offer protective immunity (MacLeod et al., 1945). Pneumococcal vaccine development however, is complicated by the existence of over 90 serotypes, each containing an antigenically distinct capsular polysaccharide (CPS). Anti-capsular antibodies are serotype specific and therefore do not cross-react with other serotypes.

Despite the large degree of antigenic variation, a relatively small number of serotypes are responsible for a majority of pneumococcal disease. As a result, in November of 1999, the FDA licensed the first pneumococcal conjugate vaccine for use in humans (Petkus, 1999). This conjugate vaccine includes CPS from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, conjugated to diphtheria CRM₁₉₇ protein. It is estimated that this formulation could prevent 86% of bacteremia cases and 83% of meningitis cases among children under 6 years (Butler et al., 1995). Other pneumococcal conjugate vaccines are being evaluated in an effort to increase protection against serotypes responsible for disease in all age groups. It is unlikely, however, that a vaccine will include > 11 individual serotypes due to antigenic competition (Dagan et al., 1997). Numerous pneumococcal conjugates have been evaluated in phase 1, 2 and 3 trials and appear to be safe and immunogenic (Eskola and Anttila, 1999). The pneumococcal conjugates have also been reported to prime for anamnestic IgG antibody responses upon boosting with polysaccharide vaccine (Ahman et al., 1998) and reduce carriage of vaccine included serotypes (Dagan et al., 1997).

Recently, bivalent meningococcal serogroups A–C poly- and oligosaccharide protein conjugate vaccine preparations have been developed (Anderson et al., 1994; Twumasi et al., 1995). Although serogroups A and C are only two of the nine meningococcal serogroups shown to cause invasive disease, they account for the majority of meningococcal strains associated with epidemics or outbreaks (Denamur et al., 1987; Pinner et al., 1992; Jackson et al., 1995). First-generation meningococcal A–C conjugates were shown to be immunogenic in Gambian (Twumasi et al., 1995) and British infants (Fairley et al., 1996). Subsequently, immunological memory was demonstrated to the serogroup C component in several studies (Leach et al., 1997; MacLennan et al., 2000). Recently, Campagne et al. (2000) reported immunogenicity and formation of immunological memory to the serogroup A component of the conjugate vaccine in Nigerian infants. In contrast to the successful development of the serogroup A–C vaccines, the development of an effective serogroup B vaccine has been complicated by the inability of the serogroup B polysaccharide to induce a significant

antibody response (Wyle et al., 1972), even when conjugated to a carrier protein (Jennings and Lugowski, 1981; Devi et al., 1997). The poor immunogenicity of serogroup B polysaccharide is attributed to immunologic tolerance induced by fetal exposure to cross-reactive, polysialated glycoproteins expressed in a variety of host tissues, such as neural cell adhesion molecules (Finne et al., 1983a,b, 1987). Studies have shown that the meningococcal A–C conjugate vaccines are efficacious and safe in adults and infants, resolving some of the problems associated with the polysaccharide vaccine. However, a considerable void in protection against endemic meningococcal disease worldwide, namely infections caused by serogroups B, Y and W-135, remains to be resolved.

In summary, conjugate vaccines are an effective strategy for converting TI-2 immune responses to TD immune responses. The success of the Hib conjugate vaccine demonstrates the impact that this formulation may have when applied to other pathogens. For conjugate vaccines to reach their full potential, a number of improvements must be made. (1) Distribution to developing countries must be improved to developing countries in an effort to confer herd immunity to a larger population. (2) The specific epidemiology of disease must be carefully investigated across broad geographic areas. This may be of particular interest to pneumococcal conjugate vaccine development where serotype distribution varies with geographic region (Makela, 2000). (3) Rational design of conjugate vaccines should be implemented. Recent data indicate that many variables affect the immune response to conjugate vaccines. Among these are chain length of the oligosaccharide, the site of conjugation of sugar moieties with respect to the T cell epitope and the density of carbohydrate hapten in the construct (Chong et al., 1997). Optimizing these factors may clearly benefit both currently licensed conjugate vaccines and those under development. The three conjugate vaccines discussed in this review serve as an example for the advantages and problems associated with this strategy. It should be noted that conjugate vaccine development against Group B Streptococci (Marques et al., 1994), *Salmonella typhi* (Szu et al., 1994), *Escherichia coli*, *Shigella sonnei* and *Shi. flexneri* is also underway (Cross et al., 1990; Cryz et al., 1995).

4. Anti-idiotypic antibodies

Conversion to a TD immune response can be accomplished by using peptides that mimic the carbohydrate antigen's structure. Aside from TD immunity, peptide antigens offer a number of advantages over carbohydrates. First, peptides are easy to produce in large amounts and require little downstream processing. Second, the vaccine is defined in chemical terms and is free of nucleic acid contamination. Third, the peptide product is stable at ambient temperatures, eliminating the need for a cold chain from manufacturing to administration. Finally, the stability of peptide products makes them suitable for application in delayed release vehicles (Babiuk, 1999). Consequently, peptide mimics of carbohydrate antigens are a viable strategy in vaccine development.

One way to identify such mimics is by using anti-idiotypic antibody (Ab2) technology. In 1974, Jerne developed what is known as the idiotypic network hypothesis. Jerne's controversial theory is based on the dual character of the antibody molecule, and its ability to regulate its own effects (Jerne, 1974). Initially, an antibody molecule binds an anti-

gen at its combining site, or paratope. The antibody molecule itself, can also act as an immunogen due to its idiotopes or antigenic structures (epitopes) present on the variable (V) portion of the molecule. A summary of Jerne's idiotypic network hypothesis is illustrated in Fig. 1. When this theory is applied to the immune system as a whole, it becomes apparent that some idiotopes produced in the body overlap with epitopes present on antigens. These mimics may possess the same biological activity as the original antigen. The first example of this biological phenomenon occurred in 1978 as Sege and Peterson developed an Ab2 against insulin (Sege and Peterson, 1978). These investigators demonstrated that this Ab2 could mimic the biological actions of insulin, regulating blood glucose level. They also showed that Ab2 mimics of insulin could bind insulin receptors on rat intestinal epithelial cells and block the uptake of insulin by these cells. Such findings demonstrate that Ab2 molecules are effective at mimicking the three-dimensional nature of various receptor–ligand interactions, and may be of great utility in drug discovery and vaccine design.

Carbohydrate specific antibodies often appear in response to aberrant glycoproteins or other determi-

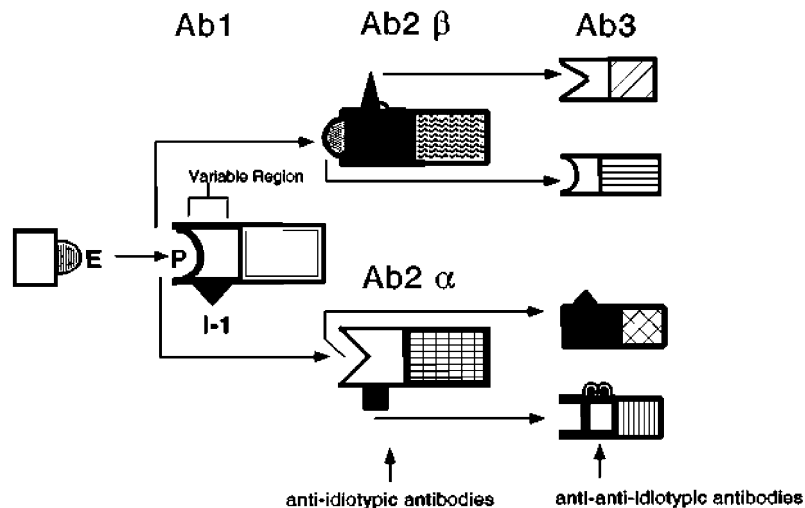


Fig. 1. Jerne's idiotypic network hypothesis. The variable region of an antibody molecule comprises many idiotopes. The combination of all idiotopes on an antibody molecule defines its "idiotypic." An epitope (E) of an antigen triggers an immune response (Ab1), and binds to a single idiotope, known as a paratope (P) on the variable region of Ab1. Idiotopes (I-1) can induce an anti-idiotypic antibody response (Ab2α) directed at regions distant from P. In addition, P can induce an anti-idiotypic antibody response (Ab2β) that contains an idiotope capable of mimicking the original antigenic epitope. Ab2α and Ab2β can induce anti-anti-idiotypic antibodies (Ab3), which may function in downregulating the immune response.

nants on cancer cells. Due to the lack of an effective CTL response against these cells, the body does not eliminate them, allowing an abnormal growth to persist. By identifying an Ab2 against such antigens, it may be possible to design a therapy or vaccine capable of redirecting the immune response against tumor cells. For example, Raychaudhuri et al. (1986) demonstrated that administering Ab2, which shared a determinant of mouse mammary tumor virus (MMTV) gp52 induced a specific, DTH reaction. Numerous other studies have investigated the role of Ab2 as a strategy to re-direct immune responses against tumor antigens (Tripathi et al., 1998).

Anti-idiotypic antibodies have been used to identify immunogenic, peptide mimics of viral, bacterial and parasitic pathogens. The utility of such mimics is being investigated against a variety of organisms ranging from hepatitis B (Rajadhyaksha et al., 1995) and *N. gonorrhoeae* (Gulati et al., 1996) to *Schistosoma mansoni* (Grzych et al., 1985). Our laboratory has employed this technique to develop a peptide mimic of the capsular polysaccharide of *N. meningitidis* serogroup C (MCPS). We have developed a monoclonal Ab2 designated 6F9 (Westerink et al., 1988) that mimics MCPS. MA b 6F9 acts as a true mimic of MCPS since it is capable of 100% inhibition of binding of human hyperimmune sera obtained from individuals convalescing from serogroup C infection to MCPS in a solid phase assay. The nature of the anti-MCPS immune response induced by mAb 6F9 was studied in Balb/c mice. The results of these studies indicate mAb 6F9 induces a T-dependent anti-MCPS response (Westerink et al., 1990). Finally, the protective efficacy of mAb 6F9 was assessed in a murine model of meningococcal infection (Westerink and Giardina, 1992). Mice immunized with mAb 6F9 were protected against infection with serogroup C meningococci. Moreover, we have completed the sequence analysis of V_L and V_H of mAb6F9 and delineated potential immunogenic regions using computer-assisted molecular modeling (Westerink et al., 1995). The analysis demonstrated the presence of several potentially immunogenic, surface exposed epitopes which included the CDR1, FR3 and a unique sequence region of the CDR3 of the V_H of 6F9. Preliminary studies demonstrated that a peptide corresponding to the unique CDR3 of 6F9, was capable of inducing an anti-MCPS antibody

response. To enhance the immunogenicity, the peptide was complexed to hydrophobic meningococcal group B (B:2a:P1.2:L3,7) outer membrane proteins referred to as proteosomes. Immunization and challenge studies with the peptide–proteosome complex in Balb/c mice indicated that the peptide induced a protective, TD antibody response. Recently, we have developed a reconstituted hu-PBL SCID mouse model which allows us to study the primary human immune response to conventional vaccines (Westerink et al., 1997). We have completed studies with the MCPS mimic peptide–proteosome preparations and were able to demonstrate that immunization with the MCPS peptide mimic induces a bactericidal human anti-MCPS response (Hutchins et al., 1999).

In summary, Ab2 can be used to identify specific peptides that mimic carbohydrate antigens. This technology may be useful for developing vaccines targeted at carbohydrate epitopes of tumor cells, viruses, bacteria and parasites. The use of Ab2, however, is not without limitations. First and foremost, the production of Ab2 and molecular modeling is costly and time consuming. Second, vaccination with Ab2 targets only a single epitope. This is in contrast to conjugate vaccines that maintain multiple polysaccharide-specific epitopes. As a result, if Ab2 technology is utilized, careful consideration must be taken to the exact epitope (preferably a protective one), which the Ab2 is intended to mimic. Third, like many peptide immunogens, Ab2 can induce idiosyncratic immunosuppression when given at high concentrations or neonatally. This flaw can be overcome, however, when Ab2 (or portions of Ab2) are coupled to immunogenic carrier proteins or given in combination with an adjuvant. Finally, there is concern that administering Ab2 may lead to harmful effects, namely, circulating rheumatoid factor or antigen–antibody complexes. Because of such limitations, the use of Ab2 has not rapidly entered into clinical trials. Their utility though, has been demonstrated in a number of animal systems against a variety of pathogens.

5. Phage display libraries

Rapid identification of peptide mimics using phage display libraries circumvents the costly and time-

consuming production of Ab2 that mimic polysaccharides. Most phage display libraries are constructed by insertion of a random peptide sequence into either the pIII or the pVIII coat protein of filamentous bacteriophage fd or m13 (Valadon and Scharff, 1996). These libraries contain over 1×10^7 clones, each of which displays a unique peptide sequence. Using monoclonal antibodies or polyclonal antisera, the phage library is screened by successive cycles of selection and amplification. A single round of affinity purification can enrich antibody-binding phage by a factor of 10^5 . As a result, antibody-binding phage can be selected from a vast background of non-binding phage (Smith and Scott, 1993). By screening a phage display library with anti-polysaccharide mAbs, it is possible to select peptides which functionally mimic the native carbohydrate antigen. Thus, TD immunity can be induced against carbohydrate antigens by immunizing with peptide mimics selected from a phage display library.

Immunogenic peptide mimics of COOH-antigens have been identified for a number of organisms. Phalipon et al. recently selected immunogenic peptide mimics from a phage display library using mAbs specific for the O-antigen (O-Ag) of *Shi. flexneri* serotype 5a lipopolysaccharide. Immunization with

the mAb-selected phage induced an O-Ag specific immune response (Phalipon et al., 1997). Similarly, Pincus et al. selected a peptide mimic of Group B streptococcal type III capsular polysaccharide (GBS) by screening a phage display library with a protective anti-GBS mAb. Immunization with the GBS-peptide mimic conjugated to a carrier molecule resulted in a significant anti-GBS antibody response (Pincus et al., 1998). Our laboratory has also used a phage display library to select immunogenic peptide mimics of capsular polysaccharides from *S. pneumoniae* serotype 4 (PPS4) (Lesinski et al., 2001), and *N. meningitidis* serogroup A (MAPS) (Grothaus et al., 2000). Because peptides alone are poor immunogens and degraded in vivo, we have found it necessary to complex the peptides to proteosomes or outer membrane proteins from *N. meningitidis* serogroup B (Zollinger et al., 1979). We have reported that immunizing mice with PPS4 mimic–proteosome complex induces a significant anti-PPS4 IgG antibody response (Fig. 2) (Lesinski et al., 2001). In addition, immunization with MAPS mimic complexed to proteosomes resulted in an anti-MAPS antibody response. Priming with MAPS peptide mimic–proteosome complex and boosting with the native polysaccharide (MAPS), resulted in a signifi-

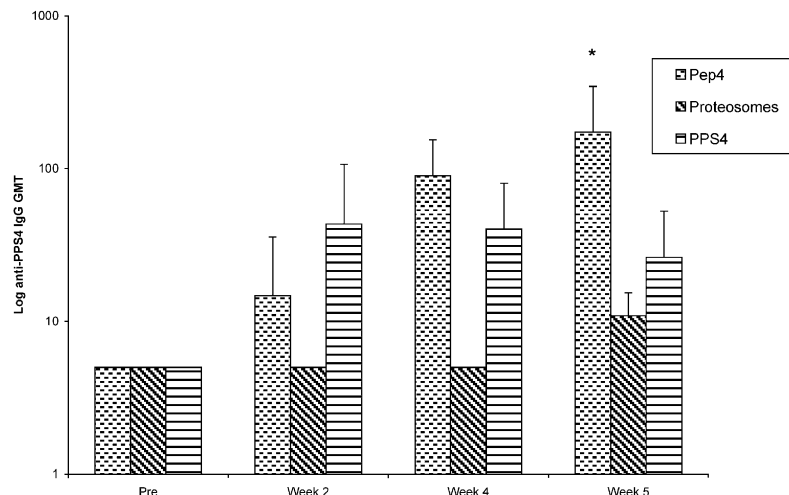


Fig. 2. Balb/c mice were immunized i.p. with 50 μ g pep4 proteosome complex on days 0, 7 and 21. Negative controls were immunized according to the same schedule with 50 μ g proteosomes. Positive control mice were immunized with 5 μ g PPS4 on day 0. Anti-PPS4 IgG antibodies in sera of individual mice were determined by ELISA assay on weeks 2, 4 and 5. The data shown are geometric mean IgG antibody titers (* indicates a significant difference at $p < 0.05$).

cantly enhanced bactericidal antibody response (Grothaus et al., 2000). These results suggest conversion to a TD immune response and formation of B cell memory.

The technology of screening phage display libraries with monoclonal antibodies is also being applied to the problematic capsular polysaccharide of *N. meningitidis* serogroup B. As previously mentioned, this polysaccharide is poorly immunogenic in humans due to cross-reactivity with fetal neural tissue. Recently, Granoff et al. (1998) have produced a panel of protective murine monoclonal antibodies that react specifically with capsular polysaccharide epitopes on meningococcal B that are distinct from host polysialic glycoproteins. These anti-capsular mAbs were used to screen a phage display library. Although the resulting peptides were capable of binding the mAbs used to select them, they failed to elicit anti-capsular antibodies. These investigators are currently screening pools of combinatorial small molecules for immunogenic mimics. This approach may lead to identification of antigens capable of eliciting protective anti-capsular antibody responses to the meningococcal serogroup B organism but avoid generating harmful autoantibodies to host polysialic acid (Moe et al., 1999).

Phage display libraries are useful and efficient tools for identifying peptide mimics, however, they have certain limitations. First, phage libraries may show a complex diversity of sequences despite negative selection, some of which are impertinent. For example, components of the biopanning process itself may favor selection of particular sequences. Components such as plastic, the antibody immobilization system or blocking agents may all influence the peptides selected (Adey et al., 1995; Caparon et al., 1996). This limitation makes it imperative that a selected peptide(s) be evaluated with respect to its biological effect, i.e. induction of anti-carbohydrate antibodies. It has been relatively common for investigators to report non-immunogenic peptide mimics capable of binding to the antibody used to select them. For example, Valadon et al. (1996) used a protective mAb, 2H1 to select peptide mimics of the capsular polysaccharide of *Cryptococcus neoformans*. Mouse immunization studies with peptide conjugated to a carrier protein indicated a strong anti-peptide antibody response. However, this peptide

was not capable of inducing cross-reactive antibodies to the *C. neoformans* polysaccharide (Valadon et al., 1998). Such findings demonstrate the difficulty of identifying mimics capable of such highly specific interactions. It is important to exclude the possibility that selected phage bind epitopes outside the antigen-binding site. This can be achieved prior to immunization by performing inhibition assays to measure binding of selected phage to antigen specific antibodies from other species. It is unlikely that epitopes other than antigen-binding regions are conserved across species. Therefore, true peptide mimics should be capable of binding antigen specific antibodies produced in a variety of species (Grothaus et al., 2000).

A second limitation is that a phage display library may not provide all possible peptides capable of binding to the antibodies. For this reason, investigators often screen multiple libraries with a panel of mAbs and compile the resulting sequences. Using this strategy, a consensus sequence can be obtained, or even a matched sequence from separate libraries. Logically, increased screening will increase the likelihood of selecting a quality mimic. Countless phage display libraries have become available within the past decade. Some are linear, and some cyclic or conformationally restrained in a variety of ways. Due to an increased interest in molecular modeling and advances in understanding the three-dimensional interaction of antigen–antibody binding, these conformational libraries have become very useful. For example, if the native conformation of the target antigen is understood, an epitope library containing appropriate conformational restraints can be engineered (Zhong et al., 1994).

Phage display libraries continue to be modified and are a useful component for selecting peptide mimics. In the future, we expect to see many new vectors for protein display other than filamentous bacteriophage. Investigators have already utilized vectors such as bacteriophage lambda (Maruyama et al., 1994), bacteriophage T4 (Sternberg and Hoess, 1995), and eukaryotic viruses (Boublik et al., 1995; Resnick et al., 1995; Turpen et al., 1995) for peptide or protein expression. In addition, databases are being established to record sequences of selected mimics from laboratories worldwide (Davies et al., 1999). This will allow for comparison of results and identi-

fication of sequence homology between antigens of a similar conformation.

6. Nucleic acid vaccines

A creative strategy for converting TI antigens to TD antigens is to combine peptide mimicry with nucleic acid vaccination. This concept involves cloning an oligodeoxynucleotide encoding a peptide mimic into a eukaryotic expression plasmid. Nucleic acid vaccines can be composed of either RNA or DNA. For the purpose of this review, the discussion will be limited to DNA vaccines. The concept of using nucleic acid as a vaccine is an approach that is in its infancy. Forty years ago, using papillomavirus nucleic acid, Ito (1960) discovered that DNA alone can transduce eukaryotic cells. In the years to follow, experiments indicated the potential of DNA plasmid constructs as delivery vehicles for the expression of genes encoding a variety of proteins (Hasan et al., 1999). Once it was discovered that gene expression in cells was long-lived following injection of plasmid DNA (Wolff et al., 1990), the potential of nucleic acid as a vaccine component was understood. In 1993, Ulmer et al. provided the first example of a DNA vaccine capable of eliciting protective immunity (Ulmer et al., 1999). In this study, intramuscular vaccination of mice with a plasmid encoding the nucleoprotein (NP) of influenza virus provided 90% protection against a lethal dose of influenza. Both NP-specific cytotoxic T cells and anti-NP antibodies were detected in vaccinated survivors. Since 1993, DNA vaccines have provided protective immunity against a variety of viral, bacterial and parasitic pathogens in a number of animal hosts (for review, see Hasan et al., 1999).

Nucleic acid vaccines offer several advantages over conventional vaccines. First, DNA vaccines are easily constructed and standardized. Second, the vaccines are extremely stable and heat resistant. Third, plasmid inserts representing the immunogen can be easily modified to increase immunogenicity, for example by adding CpG motifs. Fourth, the reduced cost of production and purification of DNA vaccines offers an economical alternative to conventional vaccines. Finally, DNA vaccines may be modified to induce lasting humoral and cellular immunity.

DNA immunization was first performed in mice by intramuscular (i.m.) injection. This is the most common route of immunization, usually targeting the quadriceps or tibialis anterior muscles of the animal. When given intramuscularly, DNA alone is sufficient to induce an immune response. Investigators often include a mild muscle degenerating agent such as the anesthetic bipivucaine-HCl or cardiotoxin. Inclusion of such agents is based on the belief that plasmid uptake may be enhanced in response to tissue damage (Davis et al., 1993; Whalen et al., 1995b). As a result, macrophages may be recruited to the site of damage or resident dendritic cells may become activated. These cells may either take up the plasmid or enhance the immune response to it (Ertl and Xiang, 1996).

More recently, DNA vaccines have been successfully administered epidermally using a “gene gun.” In this approach, DNA vaccine plasmids are coupled to gold particles less than 3 μm in diameter. Using the force from compressed helium gas, the plasmid–gold complex penetrates the cells of the skin. This results in direct transfection of cells, including epidermal Langerhans cells. Gene gun (g.g.) delivery requires only nanogram quantities of plasmid in contrast to the intramuscular route that often uses 50–100 μg of plasmid/injection (Pertmer et al., 1996; Degano et al., 1998). The efficiency of transfection is higher using a gene gun, giving a greater, more consistent immune response than i.m. injection. The choice of i.m. vs. g.g. immunization, however, can bias the immune response to either a Th1 or Th2 nature. Intramuscular immunization with plasmid DNA leads to a predominantly Th1 type immune response. In contrast, g.g. immunization normally induces a Th2 type immune response (Pertmer et al., 1996). Such studies are important for stimulating the appropriate branch of the immune system on a pathogen-specific basis.

Efforts to deliver DNA vaccines to mucosal surfaces are also being investigated. Mucosally administered vaccines have the potential to elicit both mucosal and systemic immunity. Since many pathogens invade at the mucosal surface, such a vaccine may stimulate the “first line of defense” against invasion, specifically IgA antibody. Protective immunity has been demonstrated using intranasally delivered DNA vaccine against influenza NP (Fynan et al., 1993).

Currently, efforts of many investigators are focused on more effective methods for introducing DNA vaccines to mucosal surfaces. The use of liposomes has the ability to protect DNA from rapid degradation (Gursel et al., 1999). Because evidence exists of a common mucosal immune system in the body, studies continue to focus on vaccination at alternate mucosal sites including orally and intranasally (Mason et al., 1996).

The precise mechanism by which DNA vaccines exert their effect remains under investigation. Intramuscular (i.m.) administration in particular, raises a number of questions in regard to the population of cells actually transfected. Two general theories exist to explain priming by genetic vaccines. The first theory hypothesizes that DNA vaccination results in transfection of myocytes. It is hypothesized that DNA may gain access into muscle cells through the T-tubules found only in skeletal and cardiac muscle (Wolff et al., 1992). In addition, it is believed that multiple nuclei in muscle cells may increase the probability of DNA reaching the nucleus (Dowty et al., 1995). Dupuis et al. (2000) have shown that the standard vaccine volume of 50 μ l exceeds the fluid capacity of the mouse tibialis anterior muscle, resulting in vaccine dispersion into the muscle and the interstitial space between the muscle body and the epimysial sheath. Due to the low level of MHC I expression and lack of costimulatory molecules (B7) on muscle cells, it is unlikely that myocytes act alone as Ag-presenting cells. Ag-presentation by myocytes would most likely induce anergy or tolerance rather than a robust immune response (Ertl and Xiang, 1996). Instead, it is believed that Ag is released by secretion or myocyte death. Subsequently, free antigen in the extracellular space could also be taken up by APCs or other monocytes migrating through muscle (Davis et al., 1997). This theory has been received with considerable scrutiny. Experimental evidence has downplayed the role of myocytes in the immune response to DNA vaccines. Torres et al. (1997) have demonstrated that removal of myocytes at the immunization site only 10-min post-injection, results in no effect on the humoral or cellular immune response. In a separate study, Corr et al. (1999) used bone marrow chimeras in mice, to show that the bulk of the immune response is dependent on expression of Ag by non-lymphoid tissues and

transfer to APCs. These studies provide evidence to the role of dendritic cells (DC) in enhancing the immune response to DNA vaccines.

A second theory is based on the possibility that DCs are directly transfected. DCs, however, would not contribute to the immune response unless they are activated. DCs can be activated by danger signals such as injury or infection (Matzinger, 1994). In the context of vaccination, it is possible that DC activation could result from cell damage caused by an i.m. injection (Ertl and Xiang, 1996). Results from epidermal DNA immunizations indicating direct transfection of Langerhans cells support the idea of direct DC transfection. Like DCs, Langerhans cells play an important role in antigen presentation and generation of immunity (Pertmer et al., 1996). The variety of mechanistic studies performed to date only highlight certain aspects of the immune response to DNA vaccines. It can be concluded that the mechanism of action varies with route of immunization, and that both myocytes and APCs contribute in some way to either expression, uptake or processing of antigen.

To date a majority of genetic vaccination studies have targeted protein antigens of viral, bacterial or parasitic pathogens. Because DNA vaccines act in a way that mimics natural infection, they are an ideal strategy against intracellular pathogens. Recently, DNA vaccine technology has extended to target carbohydrate antigens. As a result, DNA vaccines are now being developed against carbohydrate antigens on tumor cells and extracellular bacteria. Kieber-Emmons et al. (2000) designed a DNA vaccine encoding a peptide mimic of the carbohydrate tumor antigen Lewis Y (LeY). Intramuscular administration of this vaccine produced a Th1 type immune response characterized by IgG2a antibodies. The specific antibodies also mediated complement-dependent cytotoxicity (CDC) of a LeY expressing human tumor cell line in the presence of human complement. This study is significant because it demonstrates that peptide mimotopes constructed into DNA plasmids can produce a Th1 associated IgG2a immune response. Such an antibody response is desirable based on the observation that IgG2a antibodies are opsonizing and complement fixing.

Our laboratory has recently reported the development of a DNA vaccine against *S. pneumoniae* serotype 4 (Lesinski et al., 2001). A peptide mimic

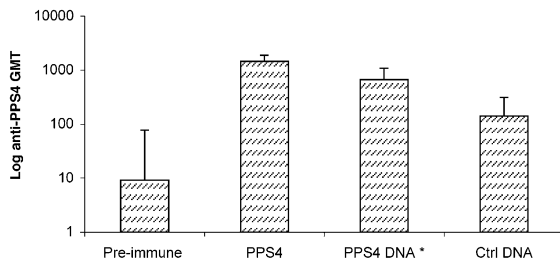


Fig. 3. The total anti-PPS4 antibody response was determined in mice following DNA immunization using an ELISA assay. Balb/c mice ($n = 8$) were immunized epidermally with 5 μg PPS4 DNA vaccine on weeks 0, 4 and 8. Negative controls ($n = 4$) were immunized with the DNA vector containing an impertinent insert. Positive controls ($n = 4$) were immunized i.p. with 5 μg PPS4. The data shown are total anti-PPS4 antibody titers (* indicates a significant difference at $p < 0.05$).

(*pep4*) of capsular polysaccharide from *S. pneumoniae* serotype 4 (PPS4) was selected from a phage display library. This mimic was capable of inducing a TD anti-PPS4 immune response in mice when complexed to proteosomes. Subsequently, an oligodeoxynucleotide encoding *pep4* was cloned into an expression vector to create a PPS4-DNA vaccine. When administered epidermally to mice, the PPS4-DNA vaccine induced PPS4-specific antibodies (Fig. 3). This study supports the idea that DNA vaccines are capable of inducing anti-carbohydrate antibodies in vivo. Due to the large number of research teams investigating peptide mimicry, it is anticipated that many more types of peptide-mimicking DNA vaccines will be developed in the near future.

7. Conclusions

We have outlined several methods to convert anti-polysaccharide immune responses from TI-2 to TD in nature. Of the strategies discussed in this review, conjugate vaccines have had the greatest impact on disease prevention to date. The remaining methods, or a combination of them, hold great promise for infectious disease or cancer prevention in future years. As our knowledge of immune system development increases, rational vaccine design is likely to follow. An increase in antibiotic resistant organisms and continued viral evolution has re-em-

phasized the importance of continuous vaccine development to the scientific community. The methods outlined in this review have clear implications for improved vaccine design, yet it is not clear what the long-range implications will be for each strategy.

References

- Adey, N.B., Mataragnon, A.H., Rider, J.E., Carter, J.M., Kay, B.K., 1995. Characterization of phage that bind plastic from phage-displayed random peptide libraries. *Gene* 156, 27–31.
- Ahman, H., Kayhty, H., Lehtonen, H., Leroy, O., Froeschle, J., Eskola, J., 1998. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to induce immunologic memory. *Pediatr. Infect. Dis. J.* 17, 211–216.
- Anderson, E.L., Bowers, T., CM, M., Kennedy, D.J., Belshe, R.B., Harakeh, H., Pais, L., Holder, P., Carlone, G.M., 1994. Safety and immunogenicity of meningococcal A and C polysaccharide conjugate vaccine in adults. *Infect. Immun.* 62, 3391–3395.
- Anonymous. (2000) Prevention and Control of Meningococcal Disease Meningococcal Disease and College Students. *MMWR* 49, 1–7.
- Artenstein, M.S., Gold, R., Zimmerly, J.G., Wyle, F.A., Branche Jr., W.C., Harkins, C., 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. *N. Engl. J. Med.* 282, 417–420.
- Avery, O.T.a.W.F.G., 1929. Chemo-immunological studies on conjugated carbohydrate proteins: II. Immunological specificity of synthetic sugar–protein antigens. *J. Exp. Med.* 50, 533–550.
- Babiuk, L.A., 1999. Broadening the approaches to developing more effective vaccines. *Vaccine* 17, 1587–1595.
- Biselli, R., Fattorossi, A., Matricardi, P.M., Nisini, R., Stroffolini, T., D'Amelio, R., 1993. Dramatic reduction of meningococcal meningitis among military recruits in Italy after introduction of specific vaccination. *Vaccine* 11, 578–581.
- Boublik, Y., Di Bonito, P., Jones, I.M., 1995. Eukaryotic virus display: engineering the major surface glycoprotein of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) for the presentation of foreign proteins on the virus surface. *Biotechnology (New York >, 13, 1079–1084* [see comments] [published erratum appears in *Biotechnology* 1995, Dec; 13 (13): 1503].
- Butler, J.C., Breiman, R.F., Lipman, H.B., Hofmann, J., Facklam, R.R., 1995. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. *J. Infect. Dis.* 171, 885–889.
- Campagne, G., Garba, A., Fabre, P., Schuchat, A., Ryall, R., Boulanger, D., Bybel, M., Carlone, G., Briantais, P., Ivanoff, B., Xerri, B., Chippaux, J.P., 2000. Safety and immunogenicity of three doses of a *Neisseria meningitidis* A + C diphtheria

- conjugate vaccine in infants from Niger. *Pediatr. Infect. Dis. J.* 19, 144–150.
- Caparon, M.H., De Ciechi, P.A., Devine, C.S., Olins, P.O., Lee, S.C., 1996. Analysis of novel streptavidin-binding peptides, identified using a phage display library, shows that amino acids external to a perfectly conserved consensus sequence and to the presented peptides contribute to binding. *Mol. Diversity* 1, 241–246.
- Chong, P., Chan, N., Kandil, A., Tripet, B., James, O., Yang, Y.P., Shi, S.P., Klein, M., 1997. A strategy for rational design of fully synthetic glycopeptide conjugate vaccines. *Infect. Immun.* 65, 4918–4925.
- Corr, M., von Damm, A., Lee, D.J., Tighe, H., 1999. In vivo priming by DNA injection occurs predominantly by antigen transfer. *J. Immunol.* 163, 4721–4727.
- Cross, A.S., Sadoff, J.C., Furer, E., Cryz Jr., S.J., 1990. *Escherichia coli* and Klebsiella vaccines and immunotherapy. *Infect. Dis. Clin. North Am.* 4, 271–282.
- Cryz Jr., S.J., Que, J.O., Cross, A.S., Furer, E., 1995. Synthesis and characterization of a polyvalent *Escherichia coli* O-poly-saccharide-toxin A conjugate vaccine. *Vaccine*, 13, 449–453.
- Dagan, R., Muallem, M., Melamed, R., Leroy, O., Yagupsky, P., 1997. Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diphtheria toxoid. *Pediatr. Infect. Dis. J.* 16, 1060–1064.
- Davies, J.M., Scealy, M., Cai, Y.P., Whisstock, J., Mackay, I.R., Rowley, M.J., 1999. Multiple alignment and sorting of peptides derived from phage-displayed random peptide libraries with polyclonal sera allows discrimination of relevant phagotopes. *Mol. Immunol.* 36, 659–667.
- Davis, H.L., Demeneix, B.A., Quantin, B., Coulombe, J., Whalen, R.G., 1993. Plasmid DNA is superior to viral vectors for direct gene transfer into adult mouse skeletal muscle. *Hum. Gene Ther.* 4, 733–740.
- Davis, H.L., Millan, C.L., Watkins, S.C., 1997. Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. *Gene Ther.* 4, 181–188.
- Degano, P., Sarphie, D.F., Bangham, C.R., 1998. Intradermal DNA immunization of mice against influenza A virus using the novel PowderJect system. *Vaccine* 16, 394–398.
- Denamur, E., Pautard, J.C., Ducroix, J.P., Masmoudi, K., Eb, F., Riou, J.Y., Orfila, J., 1987. Meningococcal disease due to group A *Neisseria meningitidis* in contacts of Mecca pilgrims. [letter] *Lancet* 2, 1211.
- Devi, S.J., Zollinger, W.D., Snoy, P.J., Tai, J.Y., Costantini, P., Norelli, F., Rappuoli, R., Frasch, C.E., 1997. Preclinical evaluation of group B *Neisseria meningitidis* and *Escherichia coli* K92 capsular polysaccharide–protein conjugate vaccines in juvenile rhesus monkeys. *Infect. Immun.* 65, 1045–1052.
- Dowty, M.E., Williams, P., Zhang, G., Hagstrom, J.E., Wolff, J.A., 1995. Plasmid DNA entry into postmitotic nuclei of primary rat myotubes. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4572–4576.
- Dupuis, M., Denis-Mize, K., Woo, C., Goldbeck, C., Selby, M.J., Chen, M., Otten, G.R., Ulmer, J.B., Donnelly, J.J., Ott, G., McDonald, D.M., 2000. Distribution of DNA vaccines determines their immunogenicity after intramuscular injection in mice. [In Process Citation] *J. Immunol.* 165, 2850–2858.
- Ertl, H.C., Xiang, Z., 1996. Novel vaccine approaches. *J. Immunol.* 156, 3579–3582.
- Eskola, J., Anttila, M., 1999. Pneumococcal conjugate vaccines. *Pediatr. Infect. Dis. J.* 18, 543–551.
- Fairley, C.K., Begg, N., Borrow, R., Fox, A.J., Jones, D.M., Cartwright, K., 1996. Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. *J. Infect. Dis.* 174, 1360–1363.
- Finne, J., Finne, U., Deagostini-Bazin, H., Goridis, C., 1983a. Occurrence of alpha 2–8 linked polysialosyl units in a neural cell adhesion molecule. *Biochem. Biophys. Res. Commun.* 112, 482–487.
- Finne, J., Leinonen, M., Makela, P.H., 1983b. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* 2, 355–357.
- Finne, J., Bitter-Suermann, D., Goridis, C., Finne, U., 1987. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. *J. Immunol.* 138, 4402–4407.
- Fynan, E.F., Webster, R.G., Fuller, D.H., Haynes, J.R., Santoro, J.C., Robinson, H.L., 1993. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proc. Natl. Acad. Sci. U. S. A.* 90, 11478–11482.
- Gold, R., Artenstein, M.S., 1971. Meningococcal infections: 2. Field trial of group C meningococcal polysaccharide vaccine in 1969–70. *Bull. World Health Organ.* 45, 279–282.
- Goldschneider, I., Gotschlich, E.C., Artenstein, M.S., 1969. Human immunity to the meningococcus: I. The role of humoral antibodies. *J. Exp. Med.* 129, 1307–1326.
- Gotschlich, E.C., Liu, T.Y., Artenstein, M.S., 1969. Human immunity to the meningococcus: III. Preparation and immunochemical properties of the Group A, Group B and Group C meningococcal polysaccharides. *J. Exp. Med.* 129, 1349–1365.
- Granoff, D.M., Holmes, S.J., Osterholm, M.T., McHugh, J.E., Lucas, A.H., Anderson, E.L., Belshe, R.B., Jacobs, J.L., Medley, F., Murphy, T.V., 1993. Induction of immunologic memory in infants primed with *Haemophilus influenzae* type b conjugate vaccines. *J. Infect. Dis.* 168, 663–671.
- Granoff, D.M., Bartoloni, A., Ricci, S., Gallo, E., Rosa, D., Ravenscroft, N., Guarnieri, V., Seid, R.C., Shan, A., Usinger, W.R., Tan, S., McHugh, Y.E., Moe, G.R., 1998. Bactericidal monoclonal antibodies that define unique meningococcal B polysaccharide epitopes that do not cross-react with human polysialic acid. *J. Immunol.* 160, 5028–5036.
- Greenwood, B.M., Bradley, A.K., Smith, A.W., Wall, R.A., 1987. Mortality from meningococcal disease during an epidemic in The Gambia, West Africa. *Trans. R. Soc. Trop. Med. Hyg.* 81, 536–538.
- Grothaus, M.C., Srivastava, N., Smithson, S.L., Kieber-Emmons, T., Williams, D.B., Carlone, G.M., Westerink, M.A., 2000. Selection of an immunogenic peptide mimic of the capsular polysaccharide of *Neisseria meningitidis* serogroup A using a peptide display library. *Vaccine* 18, 1253–1263.
- Grzych, J.M., Capron, M., Lambert, P.H., Dissous, C., Torres, S.,

- Capron, A., 1985. An anti-idiotypic vaccine against experimental schistosomiasis. *Nature* 316, 74–76.
- Gulati, S., McQuillen, D.P., Sharon, J., Rice, P.A., 1996. Experimental immunization with a monoclonal anti-idiotypic antibody that mimics the *Neisseria gonorrhoeae* lipooligosaccharide epitope 2C7. *J. Infect. Dis.* 174, 1238–1248.
- Gursel, M., Tunca, S., Ozkan, M., Ozcengiz, G., Alaeddinoglu, G., 1999. Immunoadjuvant action of plasmid DNA in liposomes. *Vaccine* 17, 1376–1383.
- Hasan, U.A., Abai, A.M., Harper, D.R., Wren, B.W., Morrow, W.J., 1999. Nucleic acid immunization: concepts and techniques associated with third generation vaccines. *J. Immunol. Methods* 229, 1–22.
- Holmes, S.J., Lucas, A.H., Osterholm, M.T., Froeschle, J.E., Granoff, D.M., 1991. Immunoglobulin deficiency and idiotype expression in children developing *Haemophilus influenzae* type b disease after vaccination with conjugate vaccine. The Collaborative Study Group. *JAMA* 266, 1960–1965.
- Hutchins, W.A., KieberEmmons, T., Carlone, G.M., Westerink, M.A.J., 1999. Human immune response to a peptide mimic of *Neisseria meningitidis* serogroup C in hu-PBMC-SCID mice. *Hybridoma* 18, 121–128.
- Ito, Y., 1960. A tumor-producing factor extracted by phenol from papillomatous tissue (Shope) of cottontail rabbits. *Virology* 12, 596–601.
- Jackson, L.A., Schuchat, A., Reeves, M.W., Wenger, J.D., 1995. Serogroup C meningococcal outbreaks in the United States. An emerging threat. [see comments] *JAMA* 273, 383–389.
- Jennings, H.J., Lugowski, C., 1981. Immunochemistry of groups A, B, and C meningococcal polysaccharide–tetanus toxoid conjugates. *J. Immunol.* 127, 1011–1018.
- Jerne, N.K., 1974. Toward a network theory of the immune system. *Ann. Immunol.* 125, 373–389.
- Jones, D., 1995. Epidemiology of meningococcal disease in Europe and in the USA. In: Cartwright, K. (Ed.), *Meningococcal Disease*. Wiley, New York, pp. 147–175.
- Kieber-Emmons, T., Monzavi-Karbassi, B., Wang, B., Luo, P., Weiner, D.B., 2000. Cutting edge: DNA immunization with minigenes of carbohydrate mimotopes induce functional anti-carbohydrate antibody response. *J. Immunol.* 165, 623–627.
- Leach, A., Twumasi, P.A., Kumah, S., Banya, W.S., Jaffar, S., Forrest, B.D., Granoff, D.M., LiButti, D.E., Carlone, G.M., Pais, L.B., Broome, C.V., Greenwood, B.M., 1997. Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide–protein conjugate vaccine. *J. Infect. Dis.* 175, 200–204.
- Lesinski, G.B., Smithson, S.L., Srivastava, N., Chen, D., Widera, G., Westerink, M.A.J., 2001. A DNA vaccine encoding a peptide mimic of *Streptococcus pneumoniae* serotype 4 capsular polysaccharide induces specific anti-carbohydrate antibodies in Balb/c mice. *Vaccine* 19, 1717–1726.
- Lindberg, A.A., 1999. Glycoprotein conjugate vaccines. *Vaccine* 17, S28–S36.
- MacLennan, J.M., Shackley, F., Heath, P.T., Deeks, J.J., Flamank, C., Herbert, M., Griffiths, H., Hatzmann, E., Goilav, C., Moxon, E.R., 2000. Safety, immunogenicity, and induction of immunologic memory by a serogroup C meningococcal conjugate vaccine in infants: a randomized controlled trial. [see comments] *JAMA* 283, 2795–2801.
- MacLeod, C.M., Hodges, R.G., Heidelberger, M., Bernhard, W.G., 1945. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. *J. Exp. Med.* 82, 445–465.
- Makela, P.H., 2000. Vaccines, coming of age after 200 years. *FEMS Microbiol. Rev.* 24, 9–20.
- Marques, M.B., Kasper, D.L., Shroff, A., Michon, F., Jennings, H.J., Wessels, M.R., 1994. Functional activity of antibodies to the group B polysaccharide of group B streptococci elicited by a polysaccharide–protein conjugate vaccine. *Infect. Immun.* 62, 1593–1599.
- Maruyama, I.N., Maruyama, H.I., Brenner, S., 1994. Lambda f00: a lambda phage vector for the expression of foreign proteins. *Proc. Natl. Acad. Sci. U. S. A.* 91, 8273–8277.
- Mason, H.S., Ball, J.M., Shi, J.J., Jiang, X., Estes, M.K., Arntzen, C.J., 1996. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5335–5340.
- Matzinger, P., 1994. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 12, 991–1045.
- McCool, T.L., Harding, C.V., Greenspan, N.S., Schreiber, J.R., 1999. B- and T-cell immune responses to pneumococcal conjugate vaccines: divergence between carrier- and polysaccharide-specific immunogenicity. *Infect. Immun.* 67, 4862–4869.
- Moe, G.R., Tan, S., Granoff, D.M., 1999. Molecular mimetics of polysaccharide epitopes as vaccine candidates for prevention of *Neisseria meningitidis* serogroup B disease. *FEMS Immunol. Med. Microbiol.* 26, 209–226.
- Neron, S., Lemieux, R., 1997. CD5 + B cell-dependent regulation of the murine T-cell independent immune response against the human blood group A antigen. *Immunol. Invest* 26, 631–647.
- Pertmer, T.M., Roberts, T.R., Haynes, J.R., 1996. Influenza virus nucleoprotein-specific immunoglobulin G subclass and cytokine responses elicited by DNA vaccination are dependent on the route of vector DNA delivery. *J. Virol.* 70, 6119–6125.
- Petkus, D., 1999. FDA Advisory Committee Recommends First Pneumococcal Conjugate Vaccine for Children. Wyeth-Ayerst Laboratories, Madison, NJ, pp. 1–2.
- Phalipon, A., Folgori, A., Arondel, J., Sgarabella, G., Fortugno, P., Cortese, R., Sansonetti, P.J., Felici, F., 1997. Induction of anti-carbohydrate antibodies by phage library-selected peptide mimics. *Eur. J. Immunol.* 27, 2620–2625.
- Pincus, S.H., Smith, M.J., Jennings, H.J., Burritt, J.B., Glee, P.M., 1998. Peptides that mimic the group B streptococcal type III capsular polysaccharide antigen. *J. Immunol.* 160, 293–298.
- Pinner, R.W., Onyango, F., Perkins, B.A., Mirza, N.B., Ngacha, D.M., Reeves, M., DeWitt, W., Njeru, E., Agata, N.N., Broome, C.V., 1992. Epidemic meningococcal disease in Nairobi, Kenya, 1989. The Kenya/Centers for Disease Control (CDC) Meningitis Study Group. *J. Infect. Dis.* 166, 359–364.
- Poland, G.A., 1999. The burden of pneumococcal disease: the role of conjugate vaccines. *Vaccine* 17, 1674–1679.
- Rajadhyaksha, M., Yang, Y.F., Thanavala, Y.M., 1995. Immunological evaluation of three generations of anti-idiotypic vaccine: study of B and T cell responses following priming with

- anti-idiotype, anti-idiotype peptide and its MAP structure. *Vaccine* 13, 1421–1426.
- Raychaudhuri, S., Saeki, Y., Fuji, H., Kohler, H., 1986. Tumor-specific idiotype vaccines: I. Generation and characterization of internal image tumor antigen. *J. Immunol.* 137, 1743–1749.
- Resnick, D.A., Smith, A.D., Gesiler, S.C., Zhang, A., Arnold, E., Arnold, G.F., 1995. Chimeras from a human rhinovirus 14-human immunodeficiency virus type 1 (HIV-1) V3 loop seroprevalence library induce neutralizing responses against HIV-1. *J. Virol.* 69, 2406–2411.
- Robbins, J.B., Austrian, R., Lee, C.J., Rastogi, S.C., Schiffman, G., Henrichsen, J., Makela, P.H., Broome, C.V., Facklam, R.R., Tiesjema, R.H. et al., 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J. Infect. Dis.* 148, 1136–1159.
- Rosenstein, N.E., Perkins, B.A., Stephens, D.S., Lefkowitz, L., Carter, M.L., Danila, R., Cieslak, P., Shutt, K.A., Popovic, T., Schuchat, A., Harrison, L.H., Reingold, A.L., 1999. The changing epidemiology of meningococcal disease in the United States, 1992–1996. *J. Infect. Dis.* 180, 1894–1901.
- Schneerson, R., Barrera, O., Sutton, A., Robbins, J.B., 1980. Preparation, characterization and immunogenicity of *Haemophilus influenzae* type B polysaccharide–protein conjugates. *J. Exp. Med.* 152, 361–376.
- Sege, K., Peterson, P.A., 1978. Use of anti-idiotypic antibodies as cell-surface receptor probes. *Proc. Natl. Acad. Sci. U. S. A.* 75, 2443–2447.
- Siber, G.R., 1994. Pneumococcal disease: prospects for a new generation of vaccines. *Science* 265, 1385–1387.
- Smith, G.P., Scott, J.K., 1993. Libraries of peptides and protein displayed on filamentous phage. *Methods Enzymol.* 217, 228–257.
- Sternberg, N., Hoess, R.H., 1995. Display of peptides and proteins on the surface of bacteriophage lambda. *Proc. Natl. Acad. Sci. U. S. A.* 92, 1609–1613.
- Szu, S.C., Taylor, D.N., Trofa, A.C., Clements, J.D., Shiloach, J., Sadoff, J.C., Bryla, D.A., Robbins, J.B., 1994. Laboratory and preliminary clinical characterization of Vi capsular polysaccharide–protein conjugate vaccines. *Infect. Immun.* 62, 4440–4444.
- Takala, A.K., Sarvas, H., Kela, E., Ronnberg, P.R., Makela, P.H., 1991. Susceptibility to invasive *Haemophilus influenzae* type b disease and the immunoglobulin G2m(n) allotype. *J. Infect. Dis.* 163, 637–639.
- Torres, C.A., Iwasaki, A., Barber, B.H., Robinson, H.L., 1997. Differential dependence on target site tissue for gene gun and intramuscular DNA immunizations. *J. Immunol.* 158, 4529–4532.
- Tripathi, P.K., Qin, H., Deng, S., Xu, C., Bhattacharya-Chatterjee, M., Foon, K.A., Chatterjee, S.K., 1998. Antigen mimicry by an anti-idiotypic antibody single chain variable fragment. *Mol. Immunol.* 35, 853–863.
- Turpen, T.H., Reinl, S.J., Charoenvit, Y., Hoffman, S.L., Fallarme, V., Grill, L.K., 1995. Malarial epitopes expressed on the surface of recombinant tobacco mosaic virus. *Biotechnology (New York)* 13, 53–57.
- Twumasi Jr., P.A., Kumah, S., Leach, A., O'Dempsey, T.J., Ceesay, S.J., Todd, J. et al., 1995. A trial of a group A plus group C meningococcal polysaccharide–protein conjugate vaccine in African infants. *J. Infect. Dis.* 171, 632–638.
- Ulmer, J.B., DeWitt, C.M., Chastain, M., Friedman, A., Donnelly, J.J., McClements, W.L., Caulfield, M.J., Bohannon, K.E., Volkin, D.B., Evans, R.K., 1999. Enhancement of DNA vaccine potency using conventional aluminum adjuvants. *Vaccine* 18, 18–28.
- Valadon, P., Scharff, M.D., 1996. Enhancement of ELISAs for screening peptides in epitope phage display libraries. *J. Immunol. Methods* 197, 171–179.
- Valadon, P., Nussbaum, G., Boyd, L.F., Margulies, D.H., Scharff, M.D., 1996. Peptide libraries define the fine specificity of anti-polysaccharide antibodies to *Cryptococcus neoformans*. *J. Mol. Biol.* 261, 11–22.
- Valadon, P., Nussbaum, G., Oh, J., Scharff, M.D., 1998. Aspects of antigen mimicry revealed by immunization with a peptide mimetic of *Cryptococcus neoformans* polysaccharide. *J. Immunol.* 161, 1829–1836.
- Westerink, M.A.J., Giardina, P.C., 1992. Anti-idiotype induced protection against *Neisseria meningitidis* serogroup C bacteremia. *Microb. Pathog.* 12, 19–26.
- Westerink, M.A.J., Campagnari, A.A., Wirth, M.A., Apicella, M.A., 1988. Development and characterization of an anti-idiotype antibody to the capsular polysaccharide of *Neisseria meningitidis* serogroup C. *Infect. Immun.* 56, 1120–1127.
- Westerink, M.A.J., Giardina, P.C., Campagnari, A.A., Apicella, M.A., 1990. The thymus dependent nature of the murine antibody response to a monoclonal anti-idiotypic antibody to the *Neisseria meningitidis* serogroup C capsular polysaccharide. *Microb. Pathog.* 8, 411–419.
- Westerink, M.A.J., Giardina, P.C., Apicella, M.A., Kieber-Emmons, T., 1995. Peptide mimicry of the meningococcal group C capsular polysaccharide. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4021–4025.
- Westerink, M.A.J., Metzger, D.W., Hutchins, W.A., Adkins, A.R., Holder, P.F., Pais, L.B., Gheesling, L.L., Carlone, G.M., 1997. Primary human immune response to *Neisseria meningitidis* serogroup C in IL-12-treated hu-PBL-SCID mice. *J. Infect. Dis.* 175, 84–90.
- Whalen, C.M., Hockin, J.C., Ryan, A., Ashton, F., 1995a. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992: emergence of a virulent clone of *Neisseria meningitidis*. *JAMA* 273, 390–394.
- Whalen, R.G., Leclerc, C., Deriaud, E., Schirmbeck, R., Reimann, J., Davis, H.L., 1995b. DNA-mediated immunization to the hepatitis B surface antigen. Activation and entrainment of the immune response. *Ann. N. Y. Acad. Sci.* 772, 64–76.
- Williams, N., 1998. T cells on the mucosal frontline. [news; comment] *Science* 280, 198–200.
- Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., Felgner, P.L., 1990. Direct gene transfer into mouse muscle in vivo. *Science* 247, 1465–1468.
- Wolff, J.A., Dowty, M.E., Jiao, S., Repetto, G., Berg, R.K., Ludtke, J.J., Williams, P., Slautterback, D.B., 1992. Expression of naked plasmids by cultured myotubes and entry of

- plasmids into T tubules and caveolae of mammalian skeletal muscle. *J. Cell Sci.* 103, 1249–1259.
- Wyle, F.A., Artenstein, M.S., Brandt, B.L., Tramont, E.C., Kasper, D.L., Altieri, P.L., Berman, S.L., Lowenthal, J.P., 1972. Immunologic response of man to group B meningococcal polysaccharide vaccines. *J. Infect. Dis.* 126, 514–521.
- Zhong, G., Smith, G.P., Berry, J., Brunham, R.C., 1994. Conformational mimicry of a chlamydial neutralization epitope on filamentous phage. *J. Biol. Chem.* 269, 24183–24188.
- Zollinger, W.D., Mandrell, R.E., Griffiss, J.M., Altieri, P., Berman, S., 1979. Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogens in man. *J. Clin. Invest.* 63, 836–848.