Aging and Disease
www.aginganddisease.org
Volume 3, Number 1; 51-67, February 2012

Review

Immune Responses to pneumococcal vaccines in children and adults: Rationale for age-specific vaccination

M.A. Julie Westerink[1], Harry W. Schroeder, Jr.[2], and Moon H. Nahm[3*]

[1] University of Toledo, Department of Medicine, Division of Infectious Diseases, Department of Medical Microbiology and Immunology and Department of Pathology,Toledo OH 43614, USA
[2] University of Alabama at Birmingham, Department of Medicine, Division of Immunology and Rheumatology, Birmingham, AL 35294, USA
[3] University of Alabama at Birmingham, Department of Pathology, Division of Laboratory Medicine, Birmingham, AL 35294, USA

[Received April 12, 2011; Revised July 19, 2011; Accepted July 19, 2011]

ABSTRACT: *Streptococcus pneumoniae* is a significant human pathogen and currently available pneumococcal vaccines are designed to elicit anti-capsule antibodies. The 23-valent polysaccharide vaccine has been used in older adults for many years whereas 7-, 10-, and 13-valent pneumococcal conjugate vaccines have only been used commonly for young children in the last decade. In addition to their high protective efficacy among children, the use of conjugate vaccines in young children has had a number of additional effects, including production of a serotype shift and providing new herd immunity to adults. The immunogenicity of both of these types of vaccines can be determined by using an ELISA assay to measure antibody levels or an opsonophagocytosis assay to assess opsonic function. As these assays have improved over time, awareness of the analytical limitations of older studies has grown. While the 23-valent vaccine is effective among young adults, it is less effective among elderly adults. Aging-associated ineffectiveness may be due to aging-dependent changes in the antibody repertoire and/or a reduction in IgM antibody production associated with aging-dependent changes in B cell subpopulations. The immunologic basis of aging-associated immune defects thus remains an active area of research.

Key words: Pneumococcus; Vaccine; Aging; B cells; Antibody repertoire

1. Pneumococcal infection epidemiology

First isolated in 1880, *Streptococcus pneumoniae* was one of the first bacteria to be characterized [1] and it is often simply referred as pneumococcus. *S. pneumoniae* is a Gram-positive bacterium with a thick polysaccharide (PS) capsule. Because this capsule dramatically increases virulence [2], it has been extensively investigated for its serologic differences. Use of polyclonal rabbit antisera led to the definition of 90 different capsule types by 1995 [3]. More recently, use of monoclonal Abs has led to the identification of several additional serotypes [4].

The pneumococcus is a major pathogen. Prior to the introduction of conjugate vaccines, it is estimated to cause 500,000 cases of pneumonia, 50,000 cases of bacteremia, and 3,000 cases of meningitis per year in the United States alone, resulting in 40,000 deaths per year [5]. The incidence of invasive pneumococcal disease (IPD), which includes bacteremia and meningitis, was 24.3 cases per 100,000 persons in 1998-1999 when pneumococcal conjugate vaccine was not yet available

***Correspondence should be addressed to:** Dr. Moon H. Nahm, University of Alabama at Birmingham, Department of Pathology, Division of Laboratory Medicine, Birmingham, AL 35294, USA. Email: nahm@uab.edu
ISSN: 2152-5250

[6]. Most episodes of pneumococcal bacteremia are secondary to pneumococcal pneumonia, with every 3 to 4 episodes of pneumonia leading to bacteremia resulting in an estimated incidence of pneumococcal pneumonia that may exceed 40-60/100,000. Pneumococcal disease incidence has a characteristic age distribution with the highest incidence seen at the extremes of age, the very young and the very old. For instance, in 1990 the incidence of bacteremic pneumococcal disease in South Carolina was found to be 160/100,000 in infants, 5/100,000 in young adults and 70/100,000 in individuals >70 years of age [7].

It is among young children that pneumococcal infections have traditionally had the most devastating impact. For example, it is estimated that 0.7-1 million of the global 1.6 million deaths due to pneumococcal infections per year occur in children [8]. In the pre-conjugate vaccine era, pneumococcus was the most common organism isolated from middle ear fluid of children with otitis media [9], accounting for approximately 50% of cases and a total of 7 million cases of otitis media per year in the United States alone [5]. Unfortunately, although routine vaccination with the conjugate vaccine has significantly reduced the incidence of otitis media caused by vaccine serotypes, pneumococci with capsular serotypes that are not found in the vaccine continue to play a major role as causative agent of otitis media [10-12]. In some regions, increase in IPD prevalence by non-vaccine types has largely replaced the reduction in the prevalence of vaccine types [13, 14].

Among elderly patients, pneumococcus is the most common organism isolated from patients with community acquired pneumonia (CAP) and accounts for at least 30% of all CAP cases [15-19]. Several reports have emphasized the importance of age in increasing the risk of acquiring pneumococcal infection [7, 20-24]. Although age is an important risk factor for IPD, several other factors play an important role in determining IPD risk for adults. These include ethnicity [25], low socioeconomic status, chronic underlying diseases such as chronic obstructive pulmonary disease (COPD), heart disease, diabetes and renal disease, and high risk behaviors such as smoking and alcohol abuse [25-29]. At particularly high risk are those individuals with recent respiratory viral illnesses (i.e., influenza, respiratory syncytial virus). Decreased mucosal clearance [30] and increased pneumococcal adherence [31] results in a significant increase in pneumococcal infections, particularly in the winter months, when these viruses tend to circulate [32]. Indeed, pneumococcal pneumonia following influenza infection was likely responsible for most of the deaths in the historical 1918 influenza epidemic [33].

Perhaps the best recognized population susceptible to pneumococcal infections is persons with immune deficiencies. For instance, children and adults who have a congenital or acquired inability to produce Abs to the capsular PS or deficiencies in early complement components or phagocytes are very susceptible to pneumococcal infections [34]. This category includes patients with selective or generalized hypogammaglobulinemia [35-37], including children with transient hypogammaglobulinemia of infancy, IgG subclass deficiency, impaired PS responsiveness, and selective IgA deficiency [38]. Children and adults with congenital, acquired or functional asplenia, such as sickle cell disease, are at significantly increased risk of fulminant IPD, emphasizing the role of the spleen in clearance of pneumococci [39, 40]. *S. pneumoniae* is a major cause of morbidity and mortality in human immunodeficiency virus (HIV) infected individuals. It is the most common bacterial respiratory pathogen encountered in the HIV-positive population and the disease is frequently complicated by bacteremia and/or recurrences [41-45]. In the era prior to the availability of highly active anti-retroviral therapy (HAART), the incidence of IPD in HIV-positive individuals was estimated to be a 100-fold higher [41] than in age-matched HIV-negative subjects. Several studies have demonstrated a significant decrease in incidence of pneumococcal disease in HIV-positive individuals in the post-HAART era [46-50]. However, despite the widespread availability of HAART, HIV-infected individuals remain at a 35-fold increased risk of IPD compared to age-matched HIV-negative controls [51].

2. Therapeutic and preventive interventions to control pneumococcal infections

Anti-capsular antisera are highly protective and were successfully used as a serotype-specific therapeutic agent before antibiotics were available [52]. With the development of antibiotics including penicillins and cephalosporins, antibiotics became the mainstay for treating pneumococcal infections. However, the rates of antimicrobial resistance in pneumococci have steadily increased [53, 54]. Moreover, despite the use of appropriate antibiotics and intensive care, for over 50 years the case fatality rate of pneumococcal bacteremia has remained at 15% to 20% in children and young adults and 30 to 40% in the elderly [55]. Furthermore, despite successful antibiotic treatment, pneumococcal meningitis can cause disabling long term sequelae.

These factors led to the development of vaccines to prevent pneumococcal infections.

The development of pneumococcal vaccines requires understanding of pathogenic mechanisms involved in pneumococcal infections. The organism colonizes the respiratory tract of healthy children and adults [56, 57] and subsequent invasion of the respiratory tract or bloodstream leads to life threatening infections such as pneumonia or sepsis [58, 59]. Antibodies (Abs) to the capsule can abrogate its shielding effect by fixing complement and opsonizing pneumococci for phagocytosis [60, 61]. This protective mechanism has been clearly illustrated by the various immune deficiencies described above with frequent and severe pneumococcal infections. Based on these observations, all presently available pneumococcal vaccines incorporate protective PS antigens.

Polysaccharide vaccines

The discovery that purified capsular PS was immunogenic led to the development of vaccines containing pooled capsular PSs of various serotypes. Several PS vaccines were developed and tested in young South African gold miners in the early 1970's. Among the miners, the 13-valent experimental PS vaccine was 82.3% efficacious against bacteremia and 78.5% against pneumococcal pneumonia [62]. Similar results were demonstrated using 6- or 12-valent versions of the vaccine [63]. Experience with these early vaccines led to the development of the 14-valent pneumococcal PS vaccine (PPV14), which was licensed in 1977. It was estimated that 68% of IPDs were caused by the 14 serotypes present in the vaccine [64]. In 1983, the presently available 23-valent PPV (PPV23) was approved, expanding serotype coverage to more than 85% of the organisms that caused IPD at that time [65]. Since its introduction, several efficacy studies have shown that in young adults the PPV is highly effective against IPD [24, 66-68].

In contrast to studies with young adults, several studies have noted a markedly decreased efficacy in the elderly [24, 69-71]. A retrospective cohort study assessed the effectiveness of the pneumococcal vaccine in 47,365 persons over 65 years of age [72]. Immunization with the pneumococcal vaccine was associated with a significant reduction in the risk of pneumococcal bacteremia with an estimated vaccine efficacy of 44%. However, the vaccine failed to reduce the risk of CAP. Several prospective randomized vaccine efficacy trials conducted among older adults have resulted in similar findings [73-76]. Suboptimal clinical efficacy likely reflects the poor immunogenicity of PPV23 in the elderly and the very young, i.e., children <2 years of age, which together represent the age groups at highest risk for IPD.

Pneumococcal conjugate vaccines

The poor immunogenicity of PPV23 in infants and the limited duration of protective antibody levels is associated with the T-independent nature of the pneumococcal capsular PS vaccine. The composition of the antibody and T cell receptor repertoires in the very young differs from that of older children and adults [77, 78]; and anti-PS antibody responses have also been associated with specific splenic B cell subsets that are not fully developed until after the age of two [79]. This latter need for a fully developed spleen may help explain both the increased susceptibility of youth and that of asplenic patients of all ages. Efforts to overcome the inherent problems of the PPV23 have led to the development of the pneumococcal conjugate vaccine (PCV). Covalent coupling of the PS to a protein carrier effectively converts the T-independent Type 2 PS into a T cell dependent antigen [80]. The potential long-term benefits or consequences of inducing a T-dependent response in place of the evolutionarily preferred T-independent process remain unknown.

Early studies demonstrated that PS conjugate vaccines can elicit a protective antibody response, recruit T cell help, and induce immunological memory in infants and young children < 2 years of age [81, 82]. PCV was prepared by conjugating pneumococcal PS to carrier proteins including the non-toxic mutant of diphtheria toxin (CRM197), as well as other proteins such as protein D of non-typeable *Haemophilus influenzae*. The conjugation process is difficult, requiring each PS to be individually conjugated and thereby limiting the number of serotypes that could be included in PCVs.

It should be mentioned that serotype distribution is both age, geographically, and temporally dependent [83-85]. These factors played an important role in the development and formulation of the conjugate vaccines. The first 7-valent conjugate vaccine (PCV7) was licensed in the United States in 2000 and included capsular PSs of serotypes 4, 6B, 9V, 14, 18C, 19F and 23F individually conjugated to a protein carrier. The selected serotypes represented approximately 80-90% of IPD [86] and 76% of otitis media [10] causing serotypes in young children in the United States. The coverage in Europe and Asia is somewhat less due to the larger contribution of serotypes 1 and 5 as causes of IPD in these parts of the world, particularly in Asia [87, 88]. PCV7 includes the five serotypes (6B, 9V, 14, 19F and

23F) representing 80% of penicillin-non-susceptible isolates at the time of marketing [86]. PCV7 has proven highly efficacious against invasive disease, with an efficacy of 97.4% [89] and it is modestly effective against pneumonia [90] in children <5 years of age. Moreover, PCV7 has reduced the risk for otitis media related physician visits by up to 40% and the need for insertion of pressure equalizing tubes (PETs) by 24.2% [91-94]. The efficacy of PCV7 on the incidence of vaccine-related serotype acute otitis media (AOM) is thought to be 65% [91]. Also, PCV7 was shown to reduce nasopharyngeal carriage of pneumococci in immunized individuals [95-99]. Clearly, introduction of PCV7 has had a major impact on the incidence of pneumococcal disease in infants and children.

In addition, PCV7 demonstrated a phenomenon called "herd immunity" by showing unexpected decrease in IPD among non-vaccinated adults [6, 100]. The decline in disease incidence was age group dependent, with a decrease of 40%, 18% and 37% in individuals between 18-49 years, 50-64 years, and those older than 65 respectively [100]. This herd immunity can be explained by the fact that old adults often acquire pneumococci from young children and that young children carry less pneumococci following vaccination. This explanation is supported by a clear temporal association between a sharp increase in IPD cases and the end-of-the-year holiday season, when the families typically get together [101].

Another interesting observation following the widespread use of the PCV7 was a shift in serotype prevalence. There was a significant decrease in diseases such as IPD and otitis media caused by vaccine serotypes, but there was an increase in diseases by non-vaccine serotypes. Amongst the most prominent of the so called replacement serotypes are 19A and 7F [102, 103]. Post-PCV7, serotype 19A became the most prevalent penicillin-nonsusceptible isolate from clinical specimens [104-107]. In Europe, serotype 1 has recently become common [108]. Another example of serotype shift was shown with a novel pneumococcal serotype 6C, which was identified in 2007 using monoclonal Abs [109]. Serotype 6C was present in the pre-PCV7 era, but had inadvertently been serotyped as 6A because of serologic similarities between serotypes 6A and 6C. Genetic studies demonstrated clear differences between them: a galactosyl transferase gene in the capsule gene locus of 6A has been replaced with a glucosyl transferase gene in 6C [4]. However, several epidemiologic investigations have found a significant increase in both carriage and IPD of 6C following the introduction and widespread use of PCV7; whereas the prevalence of 6A has decreased [110-114].

Because of these serotype shifts, the protective coverage of PCV7 was reduced. Furthermore, PCV7 did not include serotypes 1, 3, and 5 which are common in Europe, Asia and Africa. In view of this, two new PCVs (PCV10 and PCV13) have recently become available. PCV10 is licensed in Europe and contains serotypes 1, 5, and 7F in addition to the 7 serotypes in PCV7. PCV13, which became available in 2010 in the US, contains three additional serotypes: 3, 6A, and 19A [115]. These new PCVs should provide much broader coverage.

Clinical uses of vaccines and vaccination policy

Pneumococcal vaccine policies have been dictated by the epidemiology of pneumococcal infections. Moreover, individuals at highest risk of IPD fall into four general categories: children <2 years of age, individuals >65, children 2-18 years and adults 18-64 with acquired or congenital immunodeficiencies predisposing to IPD.

As young children are highly susceptible to pneumococcal infections, children are an important target population for pneumococcal vaccination. In the pre-conjugate vaccine era, vaccination recommendations with PPV23 were limited to children ≥2 years of age with increased risk for pneumococcal disease. These included children with CSF leaks, chronic cardiovascular disease, anatomical or functional asplenia, agammaglobulinemia, diabetes mellitus, sickle cell anemia and HIV [5]. After approval of the PCV7 in 2000, the Advisory Committee on Immunization Practices (ACIP) recommended routine vaccination with PCV7 of all children 2-23 months of age [86] and in 2007 these were further amended to include routine vaccination with PCV7 of all children 2-59 months of age [116]. The number of vaccine doses varies from 1 to 4 doses and is determined by age at the time of vaccination.

In 2010, PCV7 was replaced by PCV13, increasing coverage to include the so-called replacement serotypes and serotypes prevalent in countries outside of the United States. PCV13 is approved for use in children 6 weeks to 71 months of age for the prevention of IPD and otitis media. The ACIP currently recommends vaccination with PCV13 for all children 2-59 months of age and children 60-71 months of age with underlying medical conditions that increase the risk of pneumococcal disease including cochlear implants [115]. All children two years of age and older at high risk for pneumococcal disease should also receive PPV23 after completing all recommended doses of PCV13, as they may have infections caused by serotypes not included in PCV13. A single dose of PCV13 may be administered for children 6-18 years who have not received PCV13

previously and are at increased risk for invasive pneumococcal disease regardless of whether they have previously received PCV7 or PPV23 [115]. All children with asplenia, sickle cell disease, HIV, and other immunocompromising conditions should also receive a second dose of PPV23 five years after their first dose of PPV23.

Despite the fact that routine use of PCV7 among children has significantly reduced IPD in the non-vaccinated population by herd immunity [100], IPD incidence remains high but controversial in individuals ≥65 years of age and in adults with underlying medical conditions at increased risk of pneumococcal disease. One study demonstrated an increase in IPD from 1998-1999 to 2006-2007 in percentage of adult IPD in the elderly from 69% to 81% and in those with underlying medical conditions aged 18-64 from 52% to 59% [100]. However, conflicting studies demonstrate a significant decrease in IPD in these populations [117, 118]. Based on this age-related incidence of IPD, it is recommended that all individuals ≥65 years of age receive PPV23. In October of 2009, new vaccination guidelines recommend vaccination of those ≥65 years of age, 18-65 years of age with a broad array of underlying medical conditions and expanded recommendations to include all asthmatics and smokers in this age group with PPV23 [119].

Several studies have documented a significant decline in anti-pneumococcal PS antibody concentration, and likely protective efficacy, in elderly and immune compromised adults over time [24, 120-122]. It is therefore recommended that all adults 18-65 years of age with asplenia and/or immune compromise and all those vaccinated before age 65, be revaccinated 5 years after primary PPV23 vaccination. Moreover, repeated immunization with the meningococcal PS vaccine has led to immune tolerance and unresponsiveness [123]. Similar considerations have played a role in revaccination guidelines concerning PPV23. Although some studies have shown reduced responses, the most recent study from CDC found that repeat vaccination with PPV23 is safe, well-tolerated, and not associated with reduced antibody responses [124].

Invasive disease caused by *S. pneumoniae* is significantly increased in the HIV-positive population and is a major cause of morbidity and mortality. It is therefore recommended that HIV-positive individuals are vaccinated with PPV as close to the time of diagnosis as possible [125]. However, for those individuals with a $CD4^+$ cell count <200 at the time of diagnosis, the recommendations are less straight forward. Thus, HIV-infected adults with $CD4^+$ cell counts <200 "can be offered" pneumococcal vaccination [126]. Clinical evidence, however, has not confirmed efficacy in this group, although there is some potential benefit if HAART is started immediately [127]. It is currently recommended HIV-positive individuals be revaccinated with PPV23 five years post-primary vaccination [125, 126] although the clinical (or serological) benefit of revaccination is unknown.

Since PCVs are highly immunogenic and induce immunological memory in infants and children, there are attempts to use PCVs among old adults. So far, several studies in adults or the elderly have shown that PCVs do not offer an advantage in immunogenicity over the conventional PPV23 [128-134]. However, additional studies are in progress to investigate various factors such as immunization doses as well as new formulations of PCVs.

3. Monitoring immune responses to vaccination

To evaluate these different vaccines in different target populations, two different serological measures are used as surrogates of protection. One is the amount of Abs against capsular PS. The other is the capacity to opsonize pneumococci as the measure of the functional characteristics of Abs to pneumococcal capsule. While both assays are conceptually simple, the assays were found to be quite complicated and clinical studies based on non-specific assays may have produced misleading conclusions. Consequently, it is important to understand the developmental history of the pneumococcal antibody assays [135].

ELISA

When the general ELISA technology became available in the 1970's, a pneumococcal antibody ELISA was quickly developed using plastic wells coated with pneumococcal capsular PS with subsequent measurement of the amount of human IgG bound to the plastic wells. Despite the straight forward logic in the assay design, historically there had been a poor correlation between antibody concentration, as measured by ELISA, and functional antibody activity determined by opsonophagocytic assays. The poor correlation was explained when the capsular PS antigen preparations used in ELISA were found to be contaminated with a variety of cell wall components such as cell wall PS (CWPS) and other pneumococcal determinants [136, 137]. The ELISA thus measured non-functional Abs directed at other determinants in addition to the functional anti-pneumococcal PS Abs.

To improve the assay specificity, over a 20 year period the pneumococcal antibody ELISA has undergone two additional generations of improvements [135]. For

the second generation ELISA the test sera were pre-absorbed with CWPS alone. For the current third generation assay, the sera are pre-absorbed with CWPS and another capsular PS, such as PS from pneumococcal serotype 22F. This third generation ELISA has a significantly improved correlation between antibody concentration and opsonophagocytic activity [136]. It has been approved by the World Health Organization (WHO) and is now widely used [135]. Nevertheless, when one reads literature published before the development of the third generation ELISA in 2000 [135], one must be aware that the antibody amounts reported may be falsely elevated.

OPA

The opsonophagocytosis assay (OPA) is an inherently more desirable assay than ELISA as a surrogate of protection since anti-capsule Abs provide protection in vivo by opsonization. OPA was clearly preferred when pneumococcal antibody ELISA was non-specific. Even with improvements in specificity, OPA may be preferable to ELISA in certain situations. For instance, PCV7 has been shown to elicit a high level of anti-19A antibody and was expected to provide cross-protection against 19A [138, 139]. But the clinical use of PCV7 demonstrated that it is not protective against 19A [104-107] and also opsonization assays show that PCV7 does not elicit high levels of opsonic antibody to 19A [138, 139]. Perhaps the Abs have enough avidity to bind the PS, but not sufficient to opsonize pneumococci.

For the reasons above, various assays designed to measure opsonic capacity have been developed [140]. These are bioassays typically using human test serum, target bacteria, human phagocytes and rabbit complement. At the end of the assay, surviving pneumococci are enumerated. As these bioassays are technically cumbersome, they have been only rarely used in the past. However, recent improvements have resulted in a more practical OPA, which is 4-fold multiplexed and based on the classical killing type OPA [141]. In fact, the multiplexed OPA is almost as easy and fast as ELISA. In view of these changes, it is likely that there will be an increased use of OPAs in the future.

Clinical uses of ELISA and OPA

In 2005, WHO published new serological criteria to be used in consideration for licensure of new PCVs for use in infants [142, 143]. These criteria included the ability to induce an IgG anti-pneumococcal PS concentration of at least 0.35 µg/ml four weeks post-vaccination. This reference value was based on the results of three clinical efficacy trials of PCV7 conducted in infants and children [89, 142, 144-146] and the second generation ELISA (i.e., absorbed test sera with CWPS alone). The value should not be used to predict protection for an individual since the value represents estimates or threshold levels that predict protection for a group of children. Also, the trials studied protective efficacy against IPD and the value may not be useful in predicting other conditions such as nasopharyngeal carriage. Furthermore, the 0.35 µg/ml may correspond to 0.20 µg/ml if the third generation ELISA is used (i.e., the samples are absorbed with CWPS and a heterologous pneumococcal PS like serotype 22F PS) [147, 148]. The antibody concentration of 0.20-0.35 µg/ml may correspond to an opsonophagocytic titer of 1:8 for young children [142, 143].

Correlates of protection have not been defined for adult populations. The large scale protective efficacy studies necessary to achieve this goal have not been conducted in adults immunized with the PS vaccine. These studies may be difficult to conduct in the future due to the unethical nature of withholding PPV23 from target populations. Many non-vaccinated old adults have high levels of pneumococcal antibody. It is therefore generally accepted that in this population the opsonophagocytic capacity should correlate better with protection than ELISA. Although a numerical value has not been strictly assigned, higher opsonophagocytic antibody titers greater than 1:8 appear more likely to provide protection. For some serotypes, higher thresholds may be necessary.

Pneumococcal antibody ELISA was initially developed to monitor immune responses to newly produced pneumococcal vaccines. However, this assay is frequently used to assess the presence and extent of congenital or acquired immune deficiency [37]. Additional new uses of the ELISA may be to monitor therapeutically-induced immune suppression for patients that have undergone transplantation or are being treated for autoimmune diseases [149]. The blood samples for these clinical uses are generally analyzed by commercial reference laboratories, which commonly use multiplexed immunoassays based on bead array technology [150]. These multiplex assays have been extensively compared by reference laboratories with WHO ELISA using sera from old adults as well as young children. However, clinical studies based on these multiplexed immunoassays are not yet available.

While these tests have well-defined uses for vaccine development, attempts to rationalize the interpretation of the data to make clinical decisions have proved to be complex. Variables include whether to use the PPV23 or the conjugated vaccines, how to administer and utilize

them, whether to continue to use ELISAs or switch to opsonophagocytosis assays in order to measure functional responses and how to interpret the data in the context of complex clinical scenarios. As a result, the interpretation of diagnostic vaccination can sometimes lead to more questions than answers. A working group of the American Academy of Allergy Asthma and Immunology (AAAAI) is currently seeking to achieve a consensus on these issues.

4. Problems in vaccinating old adults against pneumococcus

Although efficacious in young adults, the pneumococcal PS vaccine is less effective in the population at highest risk for IPD, namely the elderly [24, 69-76]. The reason for the sub-optimal efficacy of the pneumococcal vaccine in the elderly has been the subject of numerous investigations. First, the elderly antibody response to the vaccine, as measured by RIA or IgG ELISA, has been measured and compared to that in young adults [151-162]. These studies indicate that the post-immunization anti-PS antibody concentrations in the elderly are similar to those in younger adults. Although the decrease in protective efficacy in the elderly was poorly understood based on IgG anti-PS concentrations, several mutually non-exclusive explanations are possible. First, studies performed at the CDC indicated that despite adequate IgG antibody concentrations, the elderly had a significant reduction in opsonophagocytic activity against all serotypes tested [153]. These observations were recently confirmed in two separate studies performed in elderly subjects [158, 163]. As a result, individuals with IgG antibody levels thought to be commensurate with protection may not necessarily be protected against disease. Reduced or absent functional antibody activity, as determined by opsonophagocytic or mouse protection assays, is directly related to low antibody avidity [164, 165]. Specifically, it has been demonstrated that post-vaccination sera from the elderly with low opsonophagocytic activity correlate with low IgG antibody avidity [153]. Moreover, antibody avidity appears to play a critical role in antibody function. Extensive studies with *H. influenzae* type b (Hib) anti-PS Abs have clearly demonstrated the correlation between antibody avidity, fine specificity, protective efficacy and the expression of particular V regions and clones [166-169].

Studies to define the structure-function relationship of anti-pneumococcal Abs have been performed using human monoclonal Abs [170-173], combinatorial libraries [174-176], and monoclonal Abs derived from a transgenic mouse strain reconstituted with human immunoglobulin loci (the XenoMouse model) [177, 178]. Anti-pneumococcal PS specific human monoclonal Abs of the IgM isotype generated from peripheral blood mononuclear cells (PBMC) of immunized volunteers, or from the XenoMouse model, demonstrate 97 to 100% homology to germline sequences [171, 173, 177, 178]. These mAbs used either VH3 or VH1 gene families.

Sequence analysis of human hybridomas [173] and Fabs isolated from combinatorial libraries [174, 175] of the IgG or IgA isotype indicate that they use the same restricted gene families, namely, VH3 and VH1. In contrast to the IgM Abs, the IgG and IgA Abs demonstrate high levels of somatic mutation in CDR1 and/or CDR2 indicative of a B cell memory response. Somatic mutations within canonical regions of V genes probably affect the conformation of the antigen binding groove resulting in significantly altered avidity and functional activity.

We have characterized the immunoglobulin VH gene usage of the antibody response to capsular PS of serotypes 4 and 14 in 20 young and 20 elderly adults [179]. In all volunteers, the immune response to both PSs consisted predominantly of heavy chains belonging to the VH3 gene family. There were significant differences in the variable gene repertoire between young and elderly adults. Somatic mutation occurred more frequently in sequences derived from young when compared to those derived from the elderly. When compared to young adults, aged individuals demonstarted a loss of oligoclonality in their response to pneumococcal PS of serotypes 4 and 14. The observed differences in VH repertoire, somatic mutation, and loss of oligoclonality may contribute to decreased vaccine efficacy and antibody avidity in the elderly. Studies defining the elderly immune response to pneumococcal PSs performed on a single B cell level are presently under way. These studies will allow direct correlation between antibody structure and functional activity.

A second mechanism potentially responsible for the decrease in opsonophagocytic activity may be related to the observation that the elderly produce significantly less anti-PS IgM Abs than young adults [180-184]. As IgM Abs may be available earlier during the onset of infection and they are more effective in fixing complement and opsonizing ability than IgG, the absence of IgM antibody can be significant. In a recent study, we measured the anti-PS IgG, IgM and IgA responses against serotypes 14, 18C and 19F in elderly and young adults. As previously shown, there was no difference in anti-pneumococcal PS IgG response between these age groups. The opsonophagocytic titer however, was higher in young adults compared to the elderly. Removal of IgM anti-pneumococcal PS Abs

increased the correlation coefficient between antibody concentration and opsonophagocytic activity and decreased differences in opsonophagocytic titer between young and elderly [185]. Together, these studies suggest a potentially important role for IgM Abs in generating protective immunity. Thus the current ELISA, which measures only IgG Abs, may be insufficient to truly assess the level of protection and thus ELISA assays for IgM Abs may need to be developed.

This observation is intriguing because there have been observations in the past that suggested that a human IgM memory B cell subpopulation is reduced in older adults [186, 187]. The nature of the B cell population responsible for the immune response to capsular PSs has been a controversial issue for many years. Recent studies, however, suggest that peripheral blood IgM memory B cells ($CD27^+IgM^+IgD^\pm$) may include the circulating counterpart of splenic marginal zone B cells [188, 189]. By virtue of their pre-diversified IgM antigen receptor repertoire, IgM memory B cells are capable of responding immediately, without T cell help, to TI-2 antigens [186, 187]. These IgM memory B cells are thought to form the first line of defense against encapsulated organisms.

It has been shown that persons with decreased or absent circulating IgM memory B cells, including the elderly, splenectomized persons, infants less than 2 years of age, and a subgroup of common variable immunodeficiency patients, all respond poorly to PS vaccines and are highly susceptible to infections with encapsulated organisms [79, 186, 187, 189-191]. In addition, IgM memory B cells show a significant increase in VH3 gene family representation compared to naïve B cells and are often (94.7%) somatically mutated [192]. These observations thus support the concept that IgM memory B cells are important in generating the response to TI-2 antigens.

It is, however, unlikely that IgM memory B cells are solely responsible for anti-PS antibody production. First, switched memory B cells ($CD27^+IgM^-$) secrete higher levels of anti-pneumococcal PS antibody than $CD27^+IgM^+$ memory cells following *in vitro* stimulation [193]. Second, sequence analysis of anti-pneumococcal PS Abs, 5 days post-vaccination, demonstrate a predominance of IgG and IgA Abs, which are presumably derived from switched memory cells that have undergone somatic hypermutation [174, 176, 179]. This may be due to additional PS responsive B cell subsets and/or the versatile role that IgM memory cells play in pneumococcal antibody responses, as suggested by Bossuyt and colleagues [194]. In mice, the B1b cell subset participates in IgM and isotype-switched Ab production in response to pneumococcal PS [195, 196]. However, whether a human B1b cell counterpart exists is unknown. Although the specific B cell subset(s) involved in the immune response to carbohydrate antigens in humans remains to be elucidated, it is postulated that $CD27^+IgM^+$ memory cells, significantly decreased in the elderly, contribute to the impaired immune response to pneumococcal vaccination in this population.

Although the $CD27^+IgM^+$ memory cells are thought to include effectors of the adaptive response, another subset of B cells, namely B1a cells, could be playing a significant role in the innate immunity to encapsulated organisms. In mice, B1a cells are phenotypically defined by CD5 expression. These cells, compared to conventional B2 cells, are unique in several aspects including phenotype and production of natural Abs [197, 198]. The Abs produced by B1 cells bind autoantigens and are widely cross-reactive binding a variety of bacterial antigens such as PSs and LPS [199]. Studies performed in mice demonstrate that these $CD5^+IgM^+$ B cells are spontaneous producers of natural Abs that appear to play a vital role as the first line of defense against bacterial and viral pathogens including the pneumococcus [200-203]. Moreover, lack of B1a cells increases susceptibility to pneumococcal infections [195]. Limited studies in humans have demonstrated that $CD5^+$B cells represent the majority of B cells at birth, but diminish thereafter, with low numbers present in the elderly [204-206]. The existence of a true B1a subset in humans has been hindered by the expression of CD5 on multiple B cell populations. However, a recent study by Rothstein and colleagues [207] demonstrates the human $IgM^+CD20^+CD27^+CD43^+CD5^{+/-}$ subset may be the true functional equivalent of murine B1a cells. Notably, this population would have been included in previous studies of the heterogeneous IgM^+CD27^+ "memory" population. This recently identified human B1 subset shows skewed specificity for phosphocholine and declines sharply with age. The exact role of B1 cells in increased risk of pneumococcal infection in elderly humans, however, remains to be elucidated.

Acknowledgments

This work was partially supported by a NIH grants AI-69509 to MHN, AI-081558 and AG-05978 to M.A.J.W., and AI-48115, AI-090742 and AI-090902 to HWS. We thank Dr. K. Haas in Winston-Salem, NC and Drs. K.H. Kim and H.J. Lee in Seoul, Korea for careful reading. UAB has applied for patents on serotypes 6C and 6D. MHN and HWS are employees of UAB.

References

[1] Gray BM MD (2008) The history of pneumococcal disease. In *Pneumococcal Vaccines: The Impact of Conjugate Vaccine.* (Siber G, K. K., Makel P, ed) pp. 3-17, ASM Press, Washington DC

[2] Avery OTaWFG (1929). Chemo-immunological studies on conjugated carbohydrate proteins. II. Immunological specificity of synthetic sugar-protein antigens. J Exp Med, 50: 533-550

[3] Henrichsen J (1995). Six newly recognized types of Streptococcus pneumoniae. Journal of Clinical Microbiology, 33: 2759-2762

[4] Park IH, Park S, Hollingshead SK and Nahm MH (2007). Genetic basis for the new pneumococcal serotype, 6C. Infect Immun, 75: 4482-4489

[5] (1997). Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep, 46: 1-24

[6] Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D, Facklam RR, Jorgensen JH and Schuchat A (2003). Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med, 348: 1737-1746

[7] Breiman RF, Spika JS, Navarro VJ, Darden PM and Darby CP (1990). Pneumococcal bacteremia in Charleston county, South Carolina. A decade later. Arch Intern Med, 150: 1401-1405

[8] WHO (2007). Pneumococcal conjugate vaccine for childhood immunization--WHO position paper. Wkly Epidemiol Rec, 82: 93-104

[9] Block SL, Hedrick J, Harrison CJ, Tyler R, Smith A, Findlay R and Keegan E (2002). Pneumococcal serotypes from acute otitis media in rural Kentucky. Pediatr Infect Dis J, 21: 859-865

[10] Block SL, Hedrick J, Harrison CJ, Tyler R, Smith A, Findlay R and Keegan E (2004). Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. Pediatr Infect Dis J, 23: 829-833

[11] Fletcher MA and Fritzell B (2007). Brief review of the clinical effectiveness of PREVENAR against otitis media. Vaccine, 25: 2507-2512

[12] McEllistrem MC, Adams JM, Patel K, Mendelsohn AB, Kaplan SL, Bradley JS, Schutze GE, Kim KS, Mason EO and Wald ER (2005). Acute otitis media due to penicillin-nonsusceptible *Streptococcus pneumoniae* before and after the introduction of the pneumococcal conjugate vaccine. Clin Infect Dis, 40: 1738-1744

[13] Singleton RJ, Hennessy TW, Bulkow LR, Hammitt LL, Zulz T, Hurlburt DA, Butler JC, Rudolph K and Parkinson A (2007). Invasive pneumococcal disease caused by nonvaccine serotypes among alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. JAMA, 297: 1784-1792

[14] Munoz-Almagro C, Jordan I, Gene A, Latorre C, Garcia-Garcia JJ and Pallares R (2008). Emergence of invasive pneumococcal disease caused by nonvaccine serotypes in the era of 7-valent conjugate vaccine. Clin Infect Dis, 46: 174-182

[15] Ruiz-Gonzalez A, Falguera M, Nogues A and Rubio-Caballero M (1999). Is Streptococcus pneumoniae the leading cause of pneumonia of unknown etiology? A microbiologic study of lung aspirates in consecutive patients with community-acquired pneumonia. Am J Med, 106: 385-390

[16] Venkatesan P, Gladman J and Macfarland JT (1990). A hospital study of community acquired pneumonia in the elderly. Thorax, 45: 254-258.

[17] Marrie TJ, Durant H and Yates L (1989). Community-acquired pneumonia requiring hospitalization: 5-year prospective study. Rev Infect Dis, 11: 586-599

[18] Marston BJ, Plouffe JF, File TM, Jr., Hackman BA, Salstrom SJ, Lipman HB, Kolczak MS and Breiman RF (1997). Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance Study in Ohio. The Community-Based Pneumonia Incidence Study Group. Arch Intern Med, 157: 1709-1718

[19] Bartlett JG and Mundy LM (1995). Community-acquired pneumonia. N Engl J Med, 333: 1618-1624

[20] Mufson MA, Oley G and Hughey D (1982). Pneumococcal disease in a medium-sized community in the United States. JAMA, 248: 1486-1489

[21] Schwartz JS (1982). Pneumococcal vaccine: clinical efficacy and effectiveness. Ann Intern Med, 96: 208-220

[22] Filice GA, Darby CP and Fraser DW (1980). Pneumococcal bacteremia in Charleston County, South Carolina. Am J Epidemiol, 112: 828-835

[23] Sims RV, Boyko EJ, Maislin G, Lipsky BA and Schwartz JA (1992). The role of age in susceptibility to pneumococcal infections. Age ageing, 21: 357-361

[24] Shapiro ED, Berg AT, Austrian R, Schroeder D, Parcels V, Margolis A, Adair RK and Clemens JD (1991). The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. New Engl J Med, 325: 1453-1460

[25] Watt JP, O'Brien KL, Benin AL, Whitney CG, Robinson K, Parkinson AJ, Reid R and Santosham M (2004). Invasive pneumococcal disease among Navajo adults, 1989-1998. Clin Infect Dis, 38: 496-501

[26] Dahl MS, Trollfors B, Claesson BA, Brandberg LL and Rosengren A (2001). Invasive pneumococcal infections in Southwestern Sweden: a second follow-up period of 15 years. Scand J Infect Dis, 33: 667-672

[27] Harrison LH, Dwyer DM, Billmann L, Kolczak MS and Schuchat A (2000). Invasive pneumococcal infection in Baltimore, Md: implications for immunization policy. Arch Intern Med, 160: 89-94

[28] Pastor P, Medley F and Murphy TV (1998). Invasive pneumococcal disease in Dallas County, Texas: results from population-based surveillance in 1995. Clin Infect Dis, 26: 590-595

[29] Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, Damaske B, Stefonek K, Barnes B,

Patterson J, Zell ER, Schuchat A and Whitney CG (2001). Epidemiology of invasive Streptococcus pneumoniae infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. Jama, 285: 1729-1735

[30] O'Brien KL, Walters MI, Sellman J, Quinlisk P, Regnery H, Schwartz B and Dowell SF (2000). Severe pneumococcal pneumonia in previously healthy children: the role of preceding influenza infection. Clin Infect Dis, 30: 784-789

[31] McCullers JA and Bartmess KC (2003). Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae. J Infect Dis, 187: 1000-1009

[32] Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Edwards KM, Schaffner W, Craig AS and Griffin MR (2005). Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med, 118: 285-291

[33] Morens DM, Taubenberger JK and Fauci AS (2008). Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis, 198: 962-970

[34] Sanders LA, Rijkers GT, Kuis W, Tenbergen-Meekes AJ, de Graeff-Meeder BR, Hiemstra I and Zegers BJ (1993). Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. J Allergy Clin Immunol, 91: 110-119

[35] Cunningham-Rundles C and Bodian C (1999). Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol, 92: 34-48

[36] Johnston DT, Mehaffey G, Thomas J, Young KR, Jr., Wiener H, Li J, Go RC and Schroeder HW, Jr. (2006). Increased frequency of HLA-B44 in recurrent sinopulmonary infections (RESPI). Clin Immunol, 119: 346-350

[37] Schroeder HW, Jr., Schroeder HW, 3rd and Sheikh SM (2004). The complex genetics of common variable immunodeficiency. J Investig Med, 52: 90-103

[38] Stiehm ER (2008). The four most common pediatric immunodeficiencies. J Immunotoxicol, 5: 227-234

[39] Styrt B (1990). Infection associated with asplenia: risks, mechanisms, and prevention. Am J Med, 88: 33N-42N

[40] Wara DW (1981). Host defense against Streptococcus pneumoniae: the role of the spleen. Rev Infect Dis, 3: 299-309

[41] Redd SC, Rutherford III GW, Sande MA, Lifson AR, Hadley WK, Facklam RR and Spika JS (1990). The role of human immunodeficiency virus infection in pneumococcal bacteremia in San Francisco residents. 1990, 162: 1012-1017

[42] Boschini A, Smacchia C, Di Fine M, Schiesari A, Ballarini P, Arlotti M, Gabrielli C, Castellani G, Genova M, Pantani P, Lepri AC and Rezza G (1996). Community-acquired pneumonia in a cohort of former injection drug users with and without human immunodeficiency virus infection: incidence, etiologies, and clinical aspects. Clin Infect Dis, 23: 107-113

[43] Selwyn PA, Alcabes P, Hartel D, Buono D, Schoenbaum EE, Klein RS, Davenny K and Friedland GH (1992). Clinical manifestations and predictors of disease progression in drug users with human immunodeficiency virus infection. N Engl J Med, 327: 1697-1703

[44] Schuchat A, Broome CV, Hightower A, Costa SJ and Parkin W (1991). Use of surveillance for invasive pneumococcal disease to estimate the size of the immunosuppressed HIV-infected population. Jama, 265: 3275-3279

[45] Janoff EN, Breiman RF, Daley CL and Hopewell PC (1992). Pneumococcal disease during HIV infection. Epidemiologic, clinical and immunologic perspectives. Ann Intern Med, 117: 314-324

[46] Nuorti JP, Butler JC, Gelling L, Kool JL, Reingold AL and Vugia DJ (2000). Epidemiologic relation between HIV and invasive pneumococcal disease in San Francisco County, California. Ann Intern Med, 132: 182-190

[47] Dworkin MS, Ward JW, Hanson DL, Jones JL and Kaplan JE (2001). Pneumococcal disease among human immunodeficiency virus-infected persons: incidence, risk factors, and impact of vaccination. Clin Infect Dis, 32: 794-800

[48] Grau I, Pallares R, Tubau F, Schulze MH, Llopis F, Podzamczer D, Linares J and Gudiol F (2005). Epidemiologic changes in bacteremic pneumococcal disease in patients with human immunodeficiency virus in the era of highly active antiretroviral therapy. Arch Intern Med, 165: 1533-1540

[49] Feikin DR, Feldman C, Schuchat A and Janoff EN (2004). Global strategies to prevent bacterial pneumonia in adults with HIV disease. Lancet Infect Dis, 4: 445-455

[50] Lopez-Palomo C, Martin-Zamorano M, Benitez E, Fernandez-Gutierrez C, Guerrero F, Rodriguez-Iglesias M and Giron-Gonzalez JA (2004). Pneumonia in HIV-infected patients in the HAART era: incidence, risk, and impact of the pneumococcal vaccination. J Med Virol, 72: 517-524

[51] Heffernan RT, Barrett NL, Gallagher KM, Hadler JL, Harrison LH, Reingold AL, Khoshnood K, Holford TR and Schuchat A (2005). Declining incidence of invasive Streptococcus pneumoniae infections among persons with AIDS in an era of highly active antiretroviral therapy, 1995-2000. J Infect Dis, 191: 2038-2045

[52] Cole R (1913). Treatment of pneumonia by means of specific serums. JAMA, 61: 663-666

[53] Breiman RF, Butler JC, Tenover FC, Elliott JA and Facklam RR (1994). Emergence of drug-resistant pneumococcal infections in the United States. Jama, 271: 1831-1835.

[54] Lynch JP, 3rd and Zhanel GG (2009). Streptococcus pneumoniae: does antimicrobial resistance matter? Semin Respir Crit Care Med, 30: 210-238

[55] Plouffe JF, Breiman RF and Facklam RR (1996). Bacteremia with Streptococcus pneumoniae. Implications for therapy and prevention. Franklin County Pneumonia Study Group. Jama, 275: 194-198

[56] Austrian R (1986). Some aspects of the pneumococcal carrier state. J Antimicrob Chemother, 18 Suppl A: 35-45

[57] Gray BM, Converse GM, 3rd and Dillon HC, Jr. (1980). Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis, 142: 923-933

[58] Alexander JE, Lock RA, Peeters CC, Poolman JT, Andrew PW, Mitchell TJ, Hansman D and Paton JC (1994). Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of Streptococcus pneumoniae. Infect Immun, 62: 5683-5688

[59] Butler JC, Shapiro ED and Carlone GM (1999). Pneumococcal vaccines: history, current status, and future directions. Am J Med, 107: 69S-76S

[60] Musher DL, Johnson Jr B and Watson DA (1990). Quantitative relationship between anticapsular antibody measured by enzyme-linked immunosorbent assay of radioimmunoassay and protection of mice against challenge with Streptococcus pneumoniae serotype 4. Infect Immun, 58: 3871-3876.

[61] Bruyn GA, Zegers BJ and van Furth R (1992). Mechanisms of host defense against infection with Streptococcus pneumoniae. Clin Infect Dis, 14: 251-262

[62] Austrian R, Douglas RM, Schiffman G, Coetzee AM, Koornhof HJ, Hayden-Smith S and Reid RDW (1976). Prevention of pneumococcal pneumonia by vaccination. Trans Assoc Am Physicians, 89: 184-194.

[63] Smit P, Oberholzer D, Hayden-Smith S, Koornhof HJ and Hilleman MR (1977). Protective efficacy of pneumococcal polysaccharide vaccines. Jama, 238: 2613-2616

[64] Broome CV and Facklam RR (1981). Epidemiology of clinically significant isolates of Streptococcus pneumoniae in the United States. Rev Infect Dis, 3: 277-281

[65] Robbins JB, Austrian R, Lee CJ, Rastogi SC, Schiffman G, Henrichsen J, Makela PH, Broome CV, Facklam RR, Tiesjema RH and 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

[66] Austrian R (1981). Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. Rev Infect Dis, 3: S1-17.

[67] MacLeod CM, Hodges RG, Heidelberger M and Bernhard WG (1945). Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J Exp Med, 82: 445-465.

[68] Smith AH (1976). The effects of age on the immune response to type III pneumococcal polysaccharide (SIII) and bacterial lipopolysaccharide (LPS) in BALB/c, SJL/J and C3H mice. J Immunol, 116: 469

[69] Simberkoff MS, Cross AP, Al-Ibrahim M, Baltch AL, Geiseler PJ, Nadler J, Richmond AS, Smith RP, Schiffman G, Shepard DS and et al. (1986). Efficacy of pneumococcal vaccine in high-risk patients. Results of a Veterans Administration Cooperative Study. N Engl J Med, 315: 1318-1327

[70] Hirschmann JV and Lipsky BA (1994). The pneumococcal vaccine after 15 years of use [see comments]. Arch Intern Med, 154: 373-377

[71] Fein AM, Feinsilver SH and Niederman MS (1991). Atypical manifestations of pneumonia in the elderly. Clin Chest Med, 12: 319-336

[72] Jackson LA, Neuzil KM, Yu O, Benson P, Barlow WE, Adams AL, Hanson CA, Mahoney LD, Shay DK and Thompson WW (2003). Effectiveness of pneumococcal polysaccharide vaccine in older adults. N Engl J Med, 348: 1747-1755

[73] Moore RA, Wiffen PJ and Lipsky BA (2000). Are the pneumococcal polysaccharide vaccines effective? Meta-analysis of the prospective trials. BMC Fam Pract, 1: 1

[74] Cornu C, Yzebe D, Leophonte P, Gaillat J, Boissel JP and Cucherat M (2001). Efficacy of pneumococcal polysaccharide vaccine in immunocompetent adults: a meta-analysis of randomized trials. Vaccine, 19: 4780-4790

[75] Fine MJ, Smith MA, Carson CA, Meffe F, Sankey SS, Weissfeld LA, Detsky AS and Kapoor WN (1994). Efficacy of pneumococcal vaccination in adults. A meta-analysis of randomized controlled trials. Arch Intern Med, 154: 2666-2677

[76] Watson L, Wilson BJ and Waugh N (2002). Pneumococcal polysaccharide vaccine: a systematic review of clinical effectiveness in adults. Vaccine, 20: 2166-2173

[77] George JF, Jr. and Schroeder HW, Jr. (1992). Developmental regulation of D beta reading frame and junctional diversity in T cell receptor-beta transcripts from human thymus. J Immunol, 148: 1230-1239

[78] Schroeder HW, Jr. (2006). Similarity and divergence in the development and expression of the mouse and human antibody repertoires. Dev Comp Immunol, 30: 119-135

[79] Timens W, Boes A, Rozeboom-Uiterwijk T and Poppema S (1989). Immaturity of the human splenic marginal zone in infancy. Possible contribution to the deficient infant immune response. J Immunol, 143: 3200-3206

[80] Kayhty H and Eskola J (1996). New vaccines for the prevention of pneumococcal infections. Emerg Infect Dis, 2: 289-298

[81] Black SB, Shinefield HR, Fireman B, Hiatt RA, Polen M and Vittinghoff E (1991). Efficacy in infancy of

oligosaccharide conjugate Haemophilus influenzae type b (HbOC) vaccine in a United States population of 61080 children. Pediatr Infect Dis J, 10: 97-104

[82] Stein KE (1992). Thymus-independent and thymus-dependent responses to polysaccharide antigens. J Infect Dis, 165(suppl 1): S49-52

[83] Lee CJ (1987). Bacterial capsular polysaccharides--biochemistry, immunity and vaccine. Mol Immunol, 24: 1005-1019

[84] VanDam JE, Fleer A and H S (1990). Immunogenicity and immunochemistry of Streptococcus pneumoniae capsular polysaccharides. Antonie van Leeuwenhoek, 58: 1-47

[85] Finland M and Barnes MW (1977). Changes in occurrence of capsular serotypes of Streptococcus pneumoniae at Boston City Hospital during selected years between 1935 and 1974. J Clin Microbiol, 5: 154-166

[86] (2000). Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep, 49: 1-35

[87] Hausdorff WP, Siber, G., Paradiso, P.R. (2001). Geographical differences in invasive pneumococcal disease rates and serotype frequency in young children. The Lancet, 357: 950-952

[88] Hausdorff WP, Bryant J, Paradiso PR and Siber GR (2000). Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis, 30: 100-121

[89] Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, Elvin L, Ensor KM, Hackell J, Siber G, Malinoski F, Madore D, Chang I, Kohberger R, Watson W, Austrian R and Edwards K (2000). Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J, 19: 187-195

[90] Black SB, Shinefield HR, Ling S, Hansen J, Fireman B, Spring D, Noyes J, Lewis E, Ray P, Lee J and Hackell J (2002). Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. Pediatr Infect Dis J, 21: 810-815

[91] Fireman B, Black SB, Shinefield HR, Lee J, Lewis E and Ray P (2003). Impact of the pneumococcal conjugate vaccine on otitis media. Pediatr Infect Dis J, 22: 10-16

[92] Poehling KA, Szilagyi PG, Grijalva CG, Martin SW, LaFleur B, Mitchel E, Barth RD, Nuorti JP and Griffin MR (2007). Reduction of frequent otitis media and pressure-equalizing tube insertions in children after introduction of pneumococcal conjugate vaccine. Pediatrics, 119: 707-715

[93] Grijalva CG, Poehling KA, Nuorti JP, Zhu Y, Martin SW, Edwards KM and Griffin MR (2006). National impact of universal childhood immunization with pneumococcal conjugate vaccine on outpatient medical care visits in the United States. Pediatrics, 118: 865-873

[94] Zhou F, Shefer A, Kong Y and Nuorti JP (2008). Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997-2004. Pediatrics, 121: 253-260

[95] Dagan R, Muallem, M., Melamed, R., Leroy, O., and Yagupsky, P. (1997). Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diptheria toxoid. Pediatr Infect Dis J, 16: 1060-1064

[96] Dagan R, Givon-Lavi N, Zamir O, Sikuler-Cohen M, Guy L, Janco J, Yagupsky P and Fraser D (2002). Reduction of nasopharyngeal carriage of Streptococcus pneumoniae after administration of a 9-valent pneumococcal conjugate vaccine to toddlers attending day care centers. J Infect Dis, 185: 927-936

[97] Dagan R, Melamed R, Muallem M, Piglansky L, Greenberg D, Abramson O, Mendelman PM, Bohidar N and Yagupsky P (1996). Reduction of nasopharyngeal carriage of pneumocci during the second year of life by a heptavalent conjugate pneumococcal vaccine. J Infect Dis, 174: 1271-1278

[98] Mbelle N, Huebner RE, Wasas AD, Kimura A, Chang I and Klugman KP (1999). Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. J Infect Dis, 180: 1171-1176

[99] Obaro SK, Adegbola RA, Banya WA and Greenwood BM (1996). Carriage of pneumococci after pneumococcal vaccination. Lancet, 348: 271-272.

[100] Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG and Moore MR (2010). Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis, 201: 32-41

[101] Walter ND, Taylor TH, Jr., Dowell SF, Mathis S and Moore MR (2009). Holiday spikes in pneumococcal disease among older adults. N Engl J Med, 361: 2584-2585

[102] Brueggemann AB, Pai R, Crook DW and Beall B (2007). Vaccine escape recombinants emerge after pneumococcal vaccination in the United States. PLoS Pathog, 3: e168

[103] Hicks LA, Harrison LH, Flannery B, Hadler JL, Schaffner W, Craig AS, Jackson D, Thomas A, Beall B, Lynfield R, Reingold A, Farley MM and Whitney CG (2007). Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998-2004. J Infect Dis, 196: 1346-1354

[104] Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, Farley MM, Jorgensen JH, Lexau CA, Petit S, Reingold A, Schaffner W, Thomas A, Whitney CG and Harrison LH (2009). Effect of pneumococcal

conjugate vaccine on pneumococcal meningitis. N Engl J Med, 360: 244-256

[105] Ongkasuwan J, Valdez TA, Hulten KG, Mason EO, Jr. and Kaplan SL (2008). Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. Pediatrics, 122: 34-39

[106] Pelton SI, Huot H, Finkelstein JA, Bishop CJ, Hsu KK, Kellenberg J, Huang SS, Goldstein R and Hanage WP (2007). Emergence of 19A as virulent and multidrug resistant Pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. Pediatr Infect Dis J, 26: 468-472

[107] Pichichero ME and Casey JR (2007). Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. JAMA, 298: 1772-1778

[108] Esteva C, Selva L, de Sevilla MF, Garcia-Garcia JJ, Pallares R and Munoz-Almagro C (2011). Streptococcus pneumoniae serotype 1 causing invasive disease among children in Barcelona over a 20-year period (1989-2008). Clin Microbiol Infect,

[109] Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MC and Nahm MH (2007). Discovery of a new capsular serotype (6C) within serogroup 6 of Streptococcus pneumoniae. J Clin Microbiol, 45: 1225-1233

[110] Leach AJ, Morris PS, McCallum GB, Wilson CA, Stubbs L, Beissbarth J, Jacups S, Hare K and Smith-Vaughan HC (2009). Emerging pneumococcal carriage serotypes in a high-risk population receiving universal 7-valent pneumococcal conjugate vaccine and 23-valent polysaccharide vaccine since 2001. BMC Infect Dis, 9: 121

[111] Millar EV, Pimenta FC, Roundtree A, Jackson D, Carvalho Mda G, Perilla MJ, Reid R, Santosham M, Whitney CG, Beall BW and O'Brien KL (2010). Pre- and post-conjugate vaccine epidemiology of pneumococcal serotype 6C invasive disease and carriage within Navajo and White Mountain Apache communities. Clin Infect Dis, 51: 1258-1265

[112] Nahm MH, Lin J, Finkelstein JA and Pelton SI (2009). Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. J Infect Dis, 199: 320-325

[113] Sa-Leao R, Nunes S, Brito-Avo A, Frazao N, Simoes AS, Crisostomo MI, Paulo AC, Saldanha J, Santos-Sanches I and de Lencastre H (2009). Changes in pneumococcal serotypes and antibiotypes carried by vaccinated and unvaccinated day-care centre attendees in Portugal, a country with widespread use of the seven-valent pneumococcal conjugate vaccine. Clin Microbiol Infect, 15: 1002-1007

[114] van Gils EJ, Veenhoven RH, Hak E, Rodenburg GD, Keijzers WC, Bogaert D, Trzcinski K, Bruin JP, van Alphen L, van der Ende A and Sanders EA (2010). Pneumococcal conjugate vaccination and nasopharyngeal acquisition of pneumococcal serotype 19A strains. JAMA, 304: 1099-1106

[115] Nuorti JP and Whitney CG (2010). Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep, 59: 1-18

[116] (2008). Updated recommendation from the Advisory Committee on Immunization Practices (ACIP) for use of 7-valent pneumococcal conjugate vaccine (PCV7) in children aged 24-59 months who are not completely vaccinated. MMWR Morb Mortal Wkly Rep, 57: 343-344

[117] Lexau CA, Lynfield R, Danila R, Pilishvili T, Facklam R, Farley MM, Harrison LH, Schaffner W, Reingold A, Bennett NM, Hadler J, Cieslak PR and Whitney CG (2005). Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. JAMA, 294: 2043-2051

[118] (2005). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease--United States, 1998-2003. MMWR Morb Mortal Wkly Rep, 54: 893-897

[119] (2010). Updated recommendations for prevention of invasive pneumococcal disease among adults using the 23-valent pneumococcal polysaccharide vaccine (PPSV23). MMWR Morb Mortal Wkly Rep, 59: 1102-1106

[120] Artz A, Ershler W and Longo D (2003). Pneumococcal vaccination and revaccination of older adults. Clinical Microbiology Reviews, 16: 308

[121] Torling J, Hedlund J, Konradsen HB and Ortqvist A (2003). Revaccination with the 23-valent pneumococcal polysaccharide vaccine in middle-aged and elderly persons previously treated for pneumonia. Vaccine, 22: 96-103

[122] Musher DM, Manof SB, Liss C, McFetridge RD, Marchese RD, Bushnell B, Alvarez F, Painter C, Blum MD and Silber JL (2010). Safety and antibody response, including antibody persistence for 5 years, after primary vaccination or revaccination with pneumococcal polysaccharide vaccine in middle-aged and older adults. J Infect Dis, 201: 516-524

[123] Artenstein MS and Brandt BL (1975). Immunologic hyporesponsiveness in man to group C meningococcal polysaccharide. J Immunol, 115: 5-7

[124] Hammitt LL, Bulkow LR, Singleton RJ, Pekka Nuorti J, Hummel KB, Miernyk KM, Zanis C, Whaley M, Romero-Steiner S, Butler JC, Rudolph K and Hennessy TW (2011). Repeat revaccination with 23-valent pneumococcal polysaccharide vaccine among adults aged 55-74 years living in Alaska: No evidence of hyporesponsiveness. Vaccine, 29: 2287-2295

[125] (2007). Recommended Adult Immunization Schedule - United States, October 2007 - September 2008. MMWR, 56: Q1-Q4

[126] (2008) Adult Prevention and Treatment of Opportunisitic Infections Guidelines Working Group. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents [DRAFT]. June 18, 2008; pp1-289.

[127] Hung CC, Chen MY, Hsieh SM, Hsiao CF, Sheng WH and Chang SC (2004). Clinical experience of the 23-valent capsular polysaccharide pneumococcal vaccination in HIV-1-infected patients receiving highly active antiretroviral therapy: a prospective observational study. Vaccine, 22: 2006-2012

[128] Powers DC, Anderson EL, Lottenbach K and Mink CM (1996). Reactogenicity and immunogenicity of a protein-conjugated pneumococcal oligosaccharide vaccine in older adults. J Infect Dis, 173: 1014-1018

[129] Shelly MA, Jacoby H, Riley GJ, Graves BT, Pichichero M and Treanor JJ (1997). Comparison of pneumococcal polysaccharide and CRM197-conjugated pneumococcal oligosaccharide vaccines in young and elderly adults. Infect Immun, 65: 242-247

[130] Wuorimaa T, Kayhty H, Leroy O and Eskola J (2001). Tolerability and immunogenicity of an 11-valent pneumococcal conjugate vaccine in adults. Vaccine, 19: 1863-1869

[131] Goldblatt D, Southern J, Andrews N, Ashton L, Burbidge P, Woodgate S, Pebody R and Miller E (2009). The immunogenicity of 7-valent pneumococcal conjugate vaccine versus 23-valent polysaccharide vaccine in adults aged 50-80 years. Clin Infect Dis, 49: 1318-1325

[132] Lazarus R, Clutterbuck E, Yu LM, Bowman J, Bateman EA, Diggle L, Angus B, Peto TE, Beverley PC, Mant D and Pollard AJ (2011). A randomized study comparing combined pneumococcal conjugate and polysaccharide vaccination schedules in adults. Clin Infect Dis, 52: 736-742

[133] Miernyk KM, Butler JC, Bulkow LR, Singleton RJ, Hennessy TW, Dentinger CM, Peters HV, Knutsen B, Hickel J and Parkinson AJ (2009). Immunogenicity and reactogenicity of pneumococcal polysaccharide and conjugate vaccines in alaska native adults 55-70 years of age. Clin Infect Dis, 49: 241-248

[134] Musher DM, Sampath R and Rodriguez-Barradas MC (2011). The potential role for protein-conjugate pneumococcal vaccine in adults: what is the supporting evidence? Clin Infect Dis, 52: 633-640

[135] Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, Benjamin W, Quataert SA, Hildreth S, Sikkema DJ, Kayhty H, Jonsdottir I and Nahm MH (2003). Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol, 10: 514-519

[136] Concepcion NaF, C. (2001). Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbant assay. Clinical and Diagnostic Laboratory Immunology, 8: 266-272

[137] Yu X, Sun Y, Frasch C, Concepcion N and Nahm MH (1999). Pneumococcal capsular polysaccharide preparations may contain non-C-polysaccharide contaminants that are immunogenic. Clin Diagn Lab Immunol, 6: 519-524

[138] Lee H, Nahm MH, Burton R and Kim KH (2009). Immune response in infants to the heptavalent pneumococcal conjugate vaccine against vaccine-related serotypes 6A and 19A. Clin Vaccine Immunol, 16: 376-381

[139] Yu X, Gray B, Chang S, Ward JI, Edwards KM and Nahm MH (1999). Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. J Infect Dis, 180: 1569-1576

[140] Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D and Nahm MH (2006). Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. Clin Vaccine Immunol, 13: 165-169

[141] Burton RL and Nahm MH (2006). Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. Clin Vaccine Immunol, 13: 1004-1009

[142] Jodar L, Butler J, Carlone G, Dagan R, Goldblatt D, Kayhty H, Klugman K, Plikaytis B, Siber G, Kohberger R, Chang I and Cherian T (2003). Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine, 21: 3265-3272

[143] Lee LH, Frasch CE, Falk LA, Klein DL and Deal CD (2003). Correlates of immunity for pneumococcal conjugate vaccines. Vaccine, 21: 2199-2205

[144] Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N and Pierce N (2003). A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med, 349: 1341-1348

[145] O'Brien KL, Moulton LH, Reid R, Weatherholtz R, Oski J, Brown L, Kumar G, Parkinson A, Hu D, Hackell J, Chang I, Kohberger R, Siber G and Santosham M (2003). Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. Lancet, 362: 355-361

[146] Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, Klugman KP, Madhi SA, Paradiso P and Kohberger R (2007). Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. Vaccine, 25: 3816-3826

[147] Henckaerts I, Goldblatt D, Ashton L and Poolman J (2006). Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. Clin Vaccine Immunol, 13: 356-360

[148] Poolman JT, Frasch CE, Kayhty H, Lestrate P, Madhi SA and Henckaerts I (2009). Evaluation of pneumococcal polysaccharide immunoassays using 22F adsorption step with serum samples from infants

vaccinated with conjugate vaccines. Clin Vaccine Immunol,

[149] Looney RJ, Diamond B, Holers VM, Levesque MC, Moreland L, Nahm MH and St Clair EW (2007). Guidelines for assessing immunocompetency in clinical trials for autoimmune diseases. Clin Immunol, 123: 235-243

[150] Pickering JW, Martins TB, Greer RW, Schroder MC, Astill ME, Litwin CM, Hildreth SW and Hill HR (2002). A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. Am. J. Clin. Pathol., 117: 589-596

[151] Fattal-German M, Taillandier J, Mathieu D and Bizzini B (1991). Pneumococcal vaccination of elderly individuals. Vaccine, 9: 542-544

[152] Lee H, Nahm MH and Kim KH (2010). The effect of age on the response to the pneumococcal polysaccharide vaccine. BMC Infect Dis, 10: 60

[153] Romero-Steiner S, Musher DM, Cetron MS, Pais LB, Groover JE, Fiore AE, Plikaytis BD and Carlone GM (1999). Reduction in functional antibody activity against Streptococcus pneumoniae in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity [see comments]. Clin Infect Dis, 29: 281-288

[154] Rubins JB, et al. (1998). Magnitude, Duration, Quality, and Function of Pneumococcal Vaccine Responses in Elderly Adults. Journal of Infectious Diseases, 178: 431-440

[155] Rubins JB, et al. (1999). Determination of Antibody Responses of Elderly Adults to All 23 Capsular Polysaccharides after Pneumococcal Vaccination. Infection and Immunity, 67: 5979-5984

[156] Rubins JB and Janoff EN (2001). Pneumococcal disease in the elderly: what is preventing vaccine efficacy? Drugs Aging, 18: 305-311

[157] Sankilampi U, Honkanen, P.O., Bloigu, A., Leinonen, M. (1997). Persistence of Antibodies to Pneumococcal Capsular Polysaccharide Vaccine in the Elderly. Journal of Infectious Diseases, 176: 1100-1104

[158] Schenkein JG, Park S and Nahm MH (2008). Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency. Vaccine, 26: 5521-5526

[159] Hedlund JU, Kalin ME, Örtqvist AB and Hebrichsen J (1994). Antibody response to pneumococcal vaccine in middle-aged and elderly patients recently treated for pneumonia. Arch Intern Med, 154: 1961-1965.

[160] Roghmann KJ, Tabloski PA, Bentley DW and Schiffman G (1987). Immune response of elderly adults to pneumococcus: variation by age, sex, and functional impairment. J Gerontol, 42: 265-270

[161] Sankilampi U, Honkanen PO, Bloigu A, Herva E and Leinonen M (1996). Antibody response to pneumococcal capsular polysaccharide vaccine in the elderly. J Infect Dis, 173: 387-393

[162] Musher DM, Groover JE, Rowland JM, Watson DA, Struewing JB, Baughn RE and Mufson MA (1993). Antibody to capsular polysaccharides of Streptococcus pneumoniae: prevalence, persistence, and response to revaccination. Clin Infect Dis, 17: 66-73

[163] Kim P, Chung E, Yamashita H, Hung KE, Mizoguchi A, Kucherlapati R, Fukumura D, Jain RK and Yun SH (2010). In vivo wide-area cellular imaging by side-view endomicroscopy. Nat Methods, 7: 303-305

[164] Usinger WR and Lucas AH (1999). Avidity as a determinant of the protective efficacy of human antibodies to pneumococcal capsular polysaccharides. Infect Immun, 67: 2366-2370

[165] Sun Y, Hwang Y and Nahm MH (2001). Avidity, potency, and cross-reactivity of monoclonal antibodies to pneumococcal capsular polysaccharide serotype 6B. Infect Immun, 69: 336-344

[166] Scott MG, Crimmins DL, McCourt DW, Chung G, Schable KF, Thiebe R, Quenzel EM, Zachau HG and Nahm MH (1991). Clonal characterization of the human IgG antibody repertoire to *Haemphilus influenzae* type b polysaccharide: IV. The less frequently expressed V_L are heterogenous. J Immunol, 147: 4007-4013

[167] Nahm MH, Kim KH, Anderson P, Hetherington SV and Park MK (1995). Functional capacities of clonal antibodies to Haemophilus influnzae type b polysaccharide. Infect Immunity, 63: 2989-2994

[168] Lucas AH and Granoff DM (1995). Functional differences in idiotypically defined IgG1 anti-polysaccharide antibodies elicited by vaccination with Haemophilus influenzae type B polysaccharide-protein conjugates. J Immunol, 154: 4195-4202

[169] Granoff DM, Schackelford PG, Holmes SJ, Group. TCVS and Lucas AH (1993). Variable region expression in the antibody responses of infants vaccinated with Haemophilus influenzae type b polysaccharide-protein conjugates: Description of a new l light chain associated idiotype, and the relation between idiotype expression, avidity and vaccine formulation. J Clin Invest, 91: 786-796

[170] Zhong Z, Burns T, Chang Q, Carroll M and Pirofski L (1999). Molecular and functional characteristics of a protective human monoclonal antibody to serotype 8 Streptococcus pneumoniae capsular polysaccharide. Infect Immun, 67: 4119-4127

[171] Shaw DR, Kirkham P, Schroeder HW, Jr., Roben P and Silverman GJ (1995). Structure-function studies of human monoclonal antibodies to pneumococcus type 3 polysaccharide. Ann N Y Acad Sci, 764: 370-373

[172] Park MK, Sun Y, Olander JV, Hoffmann JW and Nahm MH (1996). The repertoire of human antibodies to the carbohydrate capsule of *Streptococcus pneumoniae* 6B. J Infect Dis, 174: 75-82

[173] Baxendale HE, Davis Z, White HN, Spellerberg MB, Stevenson FK and Goldblatt D (2000). Immunogenetic analysis of the immune response to pneumococcal polysaccharide. Eur J Immunol, 30: 1214-1223

[174] Lucas AH, Moulton KD, Tang VR and Reason DC (2001). Combinatorial library cloning of human antibodies to Streptococcus pneumoniae capsular polysaccharides: variable region primary structures and

evidence for somatic mutation of Fab fragments specific for capsular serotypes 6B, 14, and 23F. Infect Immun, 69: 853-864

[175] Zhou J, Lottenbach KR, Barenkamp SJ, Lucas AH and Reason DC (2002). Recurrent variable region gene usage and somatic mutation in the human antibody response to the capsular polysaccharide of Streptococcus pneumoniae type 23F. Infect Immun, 70: 4083-4091

[176] Zhou J, Lottenbach KR, Barenkamp SJ and Reason DC (2004). Somatic hypermutation and diverse immunoglobulin gene usage in the human antibody response to the capsular polysaccharide of Streptococcus pneumoniae Type 6B. Infect Immun, 72: 3505-3514

[177] Chang Q, Zhong Z, Lees A, Pekna M and Pirofski L (2002). Structure-function relationships for human antibodies to pneumococcal capsular polysaccharide from transgenic mice with human immunoglobulin Loci. Infect Immun, 70: 4977-4986

[178] Russell ND, Corvalan JR, Gallo ML, Davis CG and Pirofski L (2000). Production of protective human antipneumococcal antibodies by transgenic mice with human immunoglobulin loci. Infect Immun, 68: 1820-1826

[179] Kolibab K, Smithson SL, Rabquer B, Khuder S and Westerink MA (2005). Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: analysis of the variable heavy chain repertoire. Infect Immun, 73: 7465-7476

[180] Ruben FL and Uhrin M (1985). Specific Immunoglobulin-Class antibody responses in the elderly before and after 14-valent pneumoncoccal vaccine. J Infect Dis, 151: 845-849

[181] Pollack M, Koles NL, Preston MJ, Brown BJ and Pier GB (1995). Functional properties of isotype-switched immunoglobulin M (IgM) and IgG monoclonal antibodies to Pseudomonas aeruginosa lipopolysaccharide. Infect Immun, 63: 4481-4488

[182] Raff HV, Bradley C, Brady W, Donaldson K, Lipsich L, Maloney G, Shuford W, Walls M, Ward P, Wolff E and et al. (1991). Comparison of functional activities between IgG1 and IgM class-switched human monoclonal antibodies reactive with group B streptococci or Escherichia coli K1. J Infect Dis, 163: 346-354

[183] Shyur SD, Raff HV, Bohnsack JF, Kelsey DK and Hill HR (1992). Comparison of the opsonic and complement triggering activity of human monoclonal IgG1 and IgM antibody against group B streptococci. J Immunol, 148: 1879-1884

[184] Taborda CP and Casadevall A (2001). Immunoglobulin M efficacy against Cryptococcus neoformans: mechanism, dose dependence, and prozone-like effects in passive protection experiments. J Immunol, 166: 2100-2107

[185] Park S and Nahm MH (2011). Older adults have a low capacity to opsonize pneumococci due to low IgM antibody response to pneumococcal vaccinations. Infect Immun, 79: 314-320

[186] Shi Y, Yamazaki T, Okubo Y, Uehara Y, Sugane K and Agematsu K (2005). Regulation of aged humoral immune defense against pneumococcal bacteria by IgM memory B cell. J Immunol, 175: 3262-3267

[187] Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, Berner R, Peters A, Boehm T, Plebani A, Quinti I and Carsetti R (2003). Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen. J Exp Med, 197: 939-945

[188] Wardemann H, Boehm T, Dear N and Carsetti R (2002). B-1a B cells that link the innate and adaptive immune responses are lacking in the absence of the spleen. J Exp Med, 195: 771-780

[189] Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, Plebani A, Kumararatne DS, Bonnet D, Tournilhac O, Tchernia G, Steiniger B, Staudt LM, Casanova JL, Reynaud CA and Weill JC (2004). Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood, 104: 3647-3654

[190] Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina A, Meini A, Plebani A, Aiuti F and Quinti I (2005). The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. J Allergy Clin Immunol, 115: 412-417

[191] Zandvoort A and Timens W (2002). The dual function of the splenic marginal zone: essential for initiation of anti-TI-2 responses but also vital in the general first-line defense against blood-borne antigens. Clin Exp Immunol, 130: 4-11

[192] Tsuiji M, Yurasov S, Velinzon K, Thomas S, Nussenzweig MC and Wardemann H (2006). A checkpoint for autoreactivity in human IgM+ memory B cell development. J Exp Med, 203: 393-400

[193] Takizawa M, Sugane K and Agematsu K (2006). Role of tonsillar IgD+CD27+ memory B cells in humoral immunity against pneumococcal infection. Hum Immunol, 67: 966-975

[194] Moens L, Wuyts M, Meyts I, De Boeck K and Bossuyt X (2008). Human memory B lymphocyte subsets fulfill distinct roles in the anti-polysaccharide and anti-protein immune response. J Immunol, 181: 5306-5312

[195] Haas KM, Poe JC, Steeber DA and Tedder TF (2005). B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to S. pneumoniae. Immunity, 23: 7-18

[196] Taillardet M, Haffar G, Mondière P, Asensio MJ, Gheit H, Burdin N, Defrance T and Genestier L (2009). The thymus-independent immunity conferred by a pneumococcal polysaccharide is mediated by long-lived plasma cells. Blood, 114: 4432-4440

[197] Berland R and Wortis HH (2002). Origins and functions of B-1 cells with notes on the role of CD5. Annu Rev Immunol, 20: 253-300

[198] Hardy RR and Hayakawa K (2001). B cell development pathways. Annu Rev Immunol, 19: 595-621
[199] Mackenzie LE, Youinou PY, Hicks R, Yuksel B, Mageed RA and Lydyard PM (1991). Auto- and polyreactivity of IgM from CD5+ and CD5- cord blood B cells. Scand J Immunol, 33: 329-335
[200] Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA and Chen J (2000). B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J Exp Med, 192: 271-280
[201] Boes M, Prodeus AP, Schmidt T, Carroll MC and Chen J (1998). A critical role of natural immunoglobulin M in immediate defense against systemic bacterial infection. J Exp Med, 188: 2381-2386
[202] Briles DE, Nahm M, Schroer K, Davie J, Baker P, Kearney J and Barletta R (1981). Antiphosphocholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 streptococcus pneumoniae. J Exp Med, 153: 694-705
[203] Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F, Hengartner H and Zinkernagel RM (1999). Control of early viral and bacterial distribution and disease by natural antibodies. Science, 286: 2156-2159
[204] Veneri D, Franchini M, Vella A, Tridente G, Semenzato G, Pizzolo G and Ortolani R (2007). Changes of human B and B-1a peripheral blood lymphocytes with age. Hematology, 12: 337-341
[205] Veneri D, Ortolani R, Franchini M, Tridente G, Pizzolo G and Vella A (2009). Expression of CD27 and CD23 on peripheral blood B lymphocytes in humans of different ages. Blood Transfus, 7: 29-34
[206] Youinou P, Jamin C and Lydyard PM (1999). CD5 expression in human B-cell populations. Immunol Today, 20: 312-316
[207] Griffin DO, Holodick NE and Rothstein TL (2011). Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20+ CD27+ CD43+ CD70. J Exp Med, 208: 67-80