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MINIREVIEW

Pneumococcal Capsular Polysaccharide Immunity in the Elderly

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ABSTRACT Immunity to pneumococcal infections is impaired in older people, and current vaccines are poorly protective against pneumococcal disease in this population. Naturally acquired immunity to pneumococcal capsular polysaccharides develops during childhood and is robust in young adults but deteriorates with advanced age. In particular, antibody levels and function are reduced in older people. Pneumococcal vaccines are recommended for people >65 years old. However, the benefits of polysaccharide and protein-conjugated vaccines in this population are small, because of both serotype replacement and incomplete protection against vaccine serotype pneumococcal disease. In this review, we overview the immune mechanisms by which naturally acquired and vaccine-induced pneumococcal capsular polysaccharide immunity declines with age, including altered colonization dynamics, reduced opsonic activity of antibodies (particularly IgM), and impaired mucosal immunity.

KEYWORDS *Streptococcus pneumoniae*, aging, colonization, immunization, immunoglobulins, mucosal immunity, pneumococcus, pneumonia

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Streptococcus pneumoniae, or the pneumococcus, is a major cause of morbidity and death in the elderly. People >65 years old experience an up to 5-fold increase in the incidence of and death due to pneumococcal community-acquired pneumonia (CAP) relative to those <65 years old (1, 2). In the United States, an estimated 600,000 episodes of pneumococcal CAP occur annually, with a total cost to society of 4.85 billion dollars (3); hospitalizations for pneumococcal CAP are predicted to increase by nearly 100% by the year 2040, with 87% of this increase accounted for by the elderly (4). In resource-rich settings, pneumococcal meningitis is becoming a disease of the elderly (5, 6) and frequently results in death or long-term sequelae, with higher mortality rates in the elderly than in any other age group (7, 8). Pneumococcal bacteremia is associated with substantial mortality rates whether in isolation or when associated with confirmed organ infection and is associated with increased incidence and mortality rates in the elderly (9, 10).

Throughout history, humans have suffered from pneumococcal disease and the pneumococcus has evolved in parallel with our immune systems (11). The first effective treatment for pneumococcal disease was passive immunotherapy, the transfer of specific immune serum from naturally immune donors or immunized animals to patients with pneumococcal pneumonia (12). Alongside antibiotic therapy, pneumococcal vaccines represent a signal success in humanity's battle against the pneumococcus. Opsonizing anti-capsular polysaccharide (anti-CPS) antibodies are a recognized correlate of protection and are common to both the natural and vaccine-induced responses to pneumococcal disease; therefore, in this review, we focus on this facet of adaptive immunity. In the first part of this review, we discuss pneumococcal colonization, naturally acquired anti-CPS immunity, and how these change during adulthood. In the second part, we focus on the response to pneumococcal vaccination in the elderly. A summary of key parameters that differ between the old and the young

TABLE 1 Summary of clinical and laboratory measurements of antipneumococcal immunity in young and old adults

Measurement	Healthy young adults	Older adults
Mucosal colonization (culture confirmed)	Occurs in up to 10% at any one time	Occurs in <5%
Colonization-associated immune boosting	Has been demonstrated	Has not been demonstrated
Circulating natural anti-CPS antibody titer	Robust	Declines with age
Circulating natural anti-CPS antibody opsonophagocytic activity	Robust	Declines profoundly with age
Anti-CPS antibody titer following vaccination	Robust	Robust
Anti-CPS antibody opsonophagocytic activity following vaccination	Robust	Declines with age
Memory B cell response to vaccination	Conflicting results between different studies; memory B cell responses may be superior in younger adults; hyporesponsiveness to multiple doses of unconjugated polysaccharide seen in all age groups	
Clinical efficacy of PPV against nonbacteremic pneumococcal pneumonia	Probable	Possible
Clinical efficacy of PPV against pneumococcal bacteremia/meningitis	Undisputed	Undisputed
Clinical efficacy of PCV against nonbacteremic pneumococcal pneumonia	Presumed but not specifically studied in young adults	Demonstrated but incomplete; hence, public health benefit disputed
Clinical efficacy of PCV against pneumococcal bacteremia/meningitis	Presumed but not specifically studied in young adults	Undisputed but limited serotype coverage

is provided in Table 1. We conclude with an overview of mucosal immunity in the elderly, a summary of important knowledge gaps, emerging strategies, and priorities for future research. Although we focus on anti-CPS antibodies, it must be emphasized that successful defense against pneumococcal invasion requires concerted input from every arm of the innate and adaptive immune systems (13, 14).

SEARCH STRATEGY

We searched PubMed for (["streptococcus pneumoniae" OR pneumococcus] AND [antibody OR humoral OR immunoglobulin] AND [aged OR aging OR elderly OR older]). No limits were applied; the search strategy was augmented by exploring the "related articles" and "cited by" fields in PubMed, as well as reviewing the reference lists of extracted articles.

THE EPIDEMIOLOGICAL, IMMUNOLOGICAL, AND PATHOLOGICAL SIGNIFICANCE OF PNEUMOCOCCAL COLONIZATION IN THE ELDERLY IS A CONTROVERSIAL TOPIC

Table 2 lists examples of studies that attempted to define the rate of pneumococcal colonization in elderly persons (defined as either >60 or >65 years old in different studies) (15–21). Much of the variation between these studies can be explained by the different sampling sites—nasopharyngeal, oropharyngeal, or saliva—and detection methods—classical culture, PCR, or some combination of the two.

Our understanding of pneumococcal colonization, disease susceptibility, and natural immunity in children, young adults, and murine models derives from traditional bacterial culture methods with nasopharyngeal specimens (22, 23). For example, salivary PCR in children can suggest rates of colonization approaching 100% (24), but this has yet to be correlated with immunological endpoints, the incidence of clinical disease, or protection against future acquisition. False-positive PCR results from other oral streptococci are also a concern, although steps have been taken to increase the test specificity in recent studies.

While studies of nasopharyngeal swab cultures from elderly adults have shown lower rates of colonization than in children (1.8 to 4.2%) (15–17), the addition of oral swabs and the combination of traditional culture and PCR can estimate rates of colonization (if it is defined as one or more samples from any site testing positive by any method) to as high as 23% in an elderly population (20) or 34% if saliva is also sampled (21).

TABLE 2 Examples of studies attempting to define the rate of pneumococcal colonization in older people by culture-based and/or molecular methods

First author (reference)	Yr	Country	No. sampled	Age (yr)	Site sampled	Analysis method	Rate of detection of pneumococci, *n* (%)
Becker-Dreps (15)	2015	USA	210	81.4 (6.3)[a]	NP[d]	Classical microbiology	4 (1.9)
Almeida (16)	2014	Portugal	3,361	74.56 (8.2)[a]	NP	Classical microbiology[g]	61 (1.8)
					OP[f]		15 (0.4)
					Overall		76 (2.3)
Flamaing (17)	2012	Belgium	503	80.3 (10.0)[a]	NP	Classical microbiology[h]	21 (4.2)
Esposito (18)	2016	Italy	417	73.97 (6.66)[a]	OP	PCR	41 (9.8)
Ansaldi (19)	2013	Italy	283	NR[e]	NP	Culture-enriched PCR	53 (18.7)
van Deursen (20)	2016	Netherlands	330	72.7 (68.7–79.0)[b]	NP	Classical microbiology	16 (5)
						PCR	32 (10)
					OP	Classical microbiology	16 (5)
						PCR	58 (18)
					Overall		75 (23)
Krone (21)	2015	Netherlands	270[c]	69 (NR)	NP	Culture-enriched PCR	13 (5)
					OP		31 (11)
					Saliva		76 (28)
					Overall		91 (34)

[a]Mean (standard deviation).
[b]Median (interquartile range).
[c]A total of 135 subjects sampled both before and after influenza-like illness. At a participant level, 65/135 (48%) tested positive on at least one occasion.
[d]NP, nasopharynx.
[e]NR, not reported.
[f]OP, oropharynx.
[g]With multiplex PCR confirmation of culture-positive specimens.
[h]A subset was also tested by *lytA* PCR. See published paper for full details.

Thus, while classical microbiological analysis of nasopharyngeal samples from elderly persons may not have as high a yield as molecular analysis of oral or salivary specimens, it has the advantage of allowing a more direct comparison with previous studies. It may be simplistic to report PCR as "more sensitive" than culture, as the clinicopathological significance of low-density, culture-negative colonization may not be equivalent to that of high-density, culture-positive colonization. Similarly, the presence of pneumococcal DNA in the oropharynx may not represent the presence of viable pneumococci in the nasopharynx.

Most importantly, high nasopharyngeal colonization rates in elderly people (23%, as defined by classical culture) have been demonstrated during an outbreak in a nursing home (25), suggesting that culture-positive nasopharyngeal colonization may be a clinically relevant measurement in the elderly.

In this review, for the reasons outlined above and to introduce an element of homogeneity when comparing studies of children, adults, older adults, and mice, we define colonization as the isolation of pneumococci from the nasopharynx by culture-based methods.

PNEUMOCOCCAL COLONIZATION AND NATURALLY ACQUIRED ANTIPNEUMOCOCCAL IMMUNITY: AN AGE-DEPENDENT PHENOMENON

The link between pneumococcal colonization (or carriage) and the subsequent development of all forms of pneumococcal disease is generally accepted, being biologically plausible and supported by experimental murine models of meningitis and studies of children with otitis media and adults with pneumonia (23, 26, 27). However, colonization may be a necessary evil; exposure to pneumococcal antigens via repeated episodes of nasopharyngeal colonization is key to acquiring and sustaining antipneumococcal immunity.

Throughout childhood, adolescence, and early adulthood, pneumococcus immunity

improves with age. Children <2 years old have high rates (>60%) of nasopharyngeal pneumococcal colonization (28, 29). Up to 15% of colonization episodes progress to clinical disease (particularly otitis media) before an immune response can clear the pathogen, which could be explained by the lack of a robust anti-CPS immune response in young children (23, 30, 31). Colonization rates fall with increasing age, and there is a corresponding reduction in pneumococcal disease (28). It seems that repeated colonization episodes lead to the development of protective immunity to the most prevalent circulating pneumococcal serotypes (anti-CPS antibodies are, in general, specific to a given serotype) (32). Following the maturation of the immune system and multiple episodes of colonization, young adults have well-functioning immune systems and established serotype-specific immunologic memory (33).

Naturally acquired immunity is multifactorial; nonspecific antipneumococcal immunity develops alongside serotype-specific immunity in children through mechanisms that have not been entirely elucidated (34). In young infants with immature anti-CPS responses, epidemiological studies have suggested that nonspecific immunity predominates (35), while serotype-specific immunity comes to the fore in older children (32). In adulthood, both epidemiologic and controlled human infection studies have suggested that serotype-specific immunity plays a major role (33, 36). We hypothesize that antipneumococcal immunity in older adults is more akin to that in young adults than to that in infants.

Young adults experience very low rates of morbidity and mortality from pneumococcal disease (e.g., 3.1 cases annually per 100,000 population versus 38.6 cases per 100,000 population in children <1 year old) (8), and their serotype-specific immunity is boosted by occasional episodes of asymptomatic colonization (33, 36, 37). However, in old age, a paradox emerges; while nasopharyngeal colonization appears to be less common in older adults (Table 2), they are at extremely high risk of pneumococcal disease.

One hypothesis suggests that the same mechanism (immunosenescence) determines increasing disease susceptibility with reduced colonization; increased circulating levels of proinflammatory cytokines ("inflammaging") could lead to clearance of colonization before a natural boosting of preexisting immunity can take place (38–40). An alternative explanation is that colonization is underdetected in this age group and that it is a precursor to disease, which cannot be prevented by the senescent elderly immune system. Mucosal immunity may be more durable than systemic humoral immunity (to be discussed in detail later)—this could explain a protection against colonization but susceptibility to invasive disease. Regardless, older adults are clearly at high risk of pneumococcal disease, and therefore their natural antipneumococcal immunity must differ from that of younger adults. Declines in both innate and adaptive immunity combined with increased rates of comorbidities all contribute to this (41), but we will focus here on antibody-mediated immunity.

NATURALLY ACQUIRED PNEUMOCOCCAL CPS ANTIBODIES: AN OVERVIEW

As outlined above, natural immunity arises following episodic colonization. Colonization leads to increased serum antipneumococcal antibody levels, which are detectable in all adults (42, 43). In this section, we will discuss their role in the control of pneumococcal disease. Anti-CPS antibodies are the most widely studied antibodies and are the direct effectors of vaccine-induced protection, and therefore we focus on these.

In addition to antibodies generated by natural colonization, others have reported on naturally arising polyvalent antibodies (often IgM) with potent antipneumococcal activity (44)—whether these antibodies are analogous to those that arise following colonization is unclear. Furthermore, it is possible that these antibodies undergo refinement and increased specification over time, stimulated by antigen presentation (45). For this review, we define naturally acquired antibodies as those that arise following pneumococcal exposure.

Anti-CPS antibodies form a key component of the adaptive immune response, binding to the pneumococcal capsule and thus opsonizing the bacteria and improving

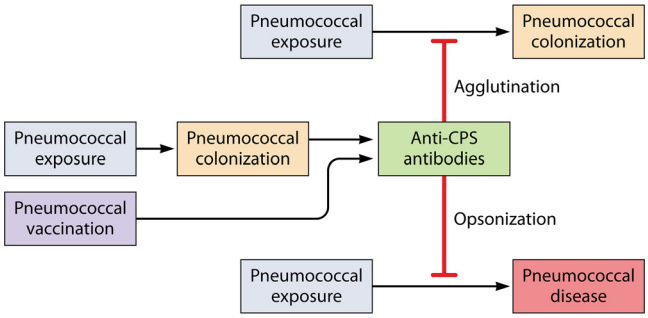

FIG 1 Anticapsular antibodies can be acquired naturally (following pneumococcal exposure, e.g., colonization, or through pneumococcal disease) or via vaccination. They facilitate pneumococcal killing via opsonization. In addition, they can prevent the development of colonization in the future—this has been shown to be mediated via agglutination in the case of antibodies induced by protein-conjugated pneumococcal vaccines.

phagocytosis and downstream killing. In addition, antibodies can promote an innate immune response by activating the classical complement pathway; in murine models, this appears to be the dominant complement pathway in antipneumococcal immunity and is mediated via natural IgM rather than IgG (46).

ANTIBODIES ARE A KEY PRODUCT OF NASOPHARYNGEAL COLONIZATION AND PROTECT AGAINST DISEASE

Antibodies are particularly effective in the control of bloodstream infections; passive transfer of human antibodies (generated following experimentally induced colonization) was protective in a murine model of lethal bacteremia (36). Passive transfer of precolonization serum from the same human volunteers conferred a lesser survival benefit. In a separate murine lethal challenge model, CD4-deficient knockout mice were able to mount a protective antibody response following experimental colonization and survive a subsequent bacteremic challenge, whereas antibody-deficient knockout mice had no survival benefit from prior colonization (47). Experimental colonization of mice also generated a protective response to subsequent pneumonia (22). However, this experiment found that all arms of the innate and adaptive immune systems are required for protection; depletion of B cells, neutrophils, or CD4 cells eliminated the protective response. This suggests that the control of mucosal disease is more complex than the control of bloodstream disease. Thus, on the basis of the evidence accumulated from a combination of murine and human challenge models, antibodies induced by pneumococcal colonization have been shown to confer protection against bacteremia and contribute to protection against pneumonia.

CLEARANCE OF COLONIZATION IS A COMPLEX PROCESS

Antibodies have an important role in protection against colonization. In mice, passive transfer of antibodies leads to agglutination of bacteria following an intranasal challenge, which causes the bacteria to clump and become more vulnerable to mucociliary clearance (48). Pneumococcal antibody-mediated agglutination has also been demonstrated in humans following vaccination with pneumococcal conjugate vaccine (PCV) (49). In that study, naturally acquired antibodies were present in the nasopharynx prior to vaccination but not at levels sufficient to induce agglutination. Murine studies have suggested that the clearance of established colonization is mediated primarily by CD4 cells and interleukin-17 (IL-17), with a possible contribution from antiprotein antibodies (50–52). Thus, it appears that anti-CPS antibodies generated during a colonization episode do not have a role in its clearance, though they may be protective against the future acquisition of colonization and subsequent development of disease. This role of anti-CPS antibodies is supported by clinical studies demonstrating the virtual elimination of vaccine serotype pneumococcal colonization in vaccinated children (53). The functional importance of anti-CPS antibodies is summarized in Fig. 1.

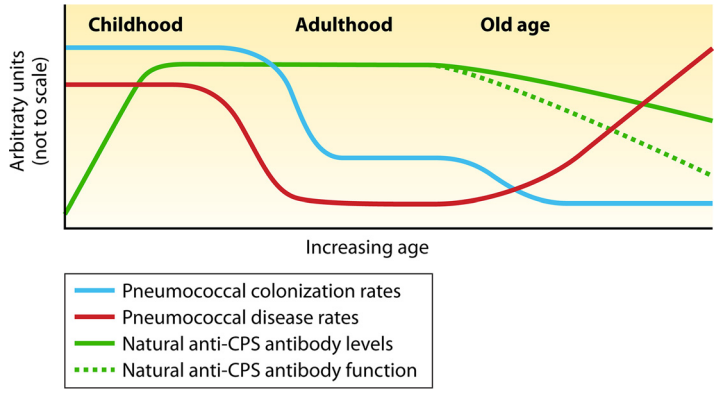

FIG 2 Schematic of pneumococcal disease rates, pneumococcal colonization rates, and pneumococcal antibody activity in different age groups. Pneumococcal colonization and disease rates are high in young children. Naturally acquired pneumococcal anti-CPS antibody levels rise with recurrent exposure. Young adults have high levels of naturally acquired antibodies, occasional episodes of colonization, and low rates of disease. In the elderly, antibody levels are low and functional activity is even lower, colonization is infrequent, and rates of pneumococcal disease increase.

WHY DOES GREATER LIFETIME EXPOSURE TO PNEUMOCOCCUS NOT LEAD TO ENHANCED PROTECTION IN THE ELDERLY?

If pneumococcal colonization leads to the generation of antibodies and these antibodies are protective against pneumococcus reacquisition, then elderly people should be particularly well protected against pneumococcal disease. Clearly, this is not the case, and several explanations have been proposed. Vaccine-induced antipneumococcal antibodies wane over time and require booster vaccines to maintain protective levels. Perhaps colonization-induced antibodies may require boosting by regular episodes of colonization (36), and this is too infrequent in elderly populations for boosting to occur. Otherwise, the defect in antibody-mediated immunity lies either with the B cells responsible for secreting the antibodies or with the antibodies themselves. Taking a wider view, T cell control of B cell responses and antibody secretion could also be implicated (41), as could alteration of neutrophil function with age (54); however, to save space, we will confine our attention to B cells and antibodies.

B CELL POPULATIONS ARE ALTERED IN OLDER PEOPLE

IgM memory B cells, which function in a T cell-independent manner, are a key component of antipneumococcal defenses (45). A study comparing healthy elderly volunteers with younger adults found that IgM memory B cells are less abundant in the elderly (55). In addition, aged IgM memory B cells were determined to be functionally inferior, with a reduced capacity for antibody secretion and plasma cell differentiation. Pneumococcal polysaccharide vaccination of elderly volunteers led to some improvement in IgM levels and IgM memory B cell percentages, but not to the same degree as in younger persons. B1 cells are another potential culprit; these cells are responsible for producing naturally acquired anti-CPS antibodies (while T cell-dependent adaptive antibodies are generated by B2 cells). Levels of B1 cells are reduced in the elderly (reviewed in reference 56). This is an emerging field, and there is a dearth of human studies relevant to this topic outside the context of vaccination—we will explore this in a later section.

ANTIBODIES DECLINE AND LOSE FUNCTIONAL EFFICACY WITH AGE

Figure 2 shows a schematic of anti-CPS antibody levels and function at different ages relative to rates of pneumococcal colonization and disease. Population-based studies have shown that natural anti-CPS IgG and IgM levels fall with age (42, 57, 58). Antibody function, i.e., opsonic activity, can vary markedly between individuals; populations with high rates of pneumococcal colonization and disease have higher serum opsonic activity than lower-risk populations, even when matched for age and

antibody level (59). For this reason, opsonophagocytic killing activity is accepted as a better correlate of protection than antibody levels (60). It is therefore of greater importance that the naturally acquired anti-CPS antibodies of older people have less opsonic activity than those of young people. In one study, the concentration of natural serotype-specific IgG required for 50% opsonic killing was up to twice as high in an unvaccinated elderly population as in a young population—differences in IgG function between young and old were even more substantial than differences in concentrations (54). Similar, though less pronounced, differences were seen in IgM. The authors noted that serotype-specific IgM concentrations and opsonic activity were poorly correlated, unlike those of IgG. When the declines in antibody level and function are combined, this strongly suggests that antibody defects are responsible for (or at least contribute to) the age-related increase in vulnerability to pneumococcal infection.

Impaired opsonic functionality relative to antibody levels is seen in immunosuppression secondary to a wide variety of etiologies. Although the situation is not directly comparable to that of the elderly, it is notable that anti-CPS IgG levels in HIV-infected individuals (who have high rates of pneumococcal colonization, as well as disease) have been shown to be higher than those in non-HIV-infected persons, but with reduced opsonic activity (61).

An observational study provides some clinical context and supports the hypothesis that reduced opsonic functionality in anti-CPS antibodies is a risk factor for pneumococcal disease in the elderly. Serum samples from patients in the acute and convalescent stages of various types of pneumococcal disease were compared with those from age-matched controls (62). Only 27% of the persons with pneumococcal disease had IgG to their infecting serotype at the time of presentation (compared to 37% of the controls and 42% of the colonized persons). Furthermore, acute antibodies from infected persons had significantly lower opsonic activity than those from controls or colonized persons and were less protective via passive transfer in a lethal murine challenge model (20% versus 100% survival). Sixty-two percent of convalescent-phase serum samples had detectable IgG following pneumococcal disease, which demonstrated good function in >50% of the patients. Important limitations of this study include substantial loss to follow-up between the acute and convalescent phases, no reporting of ages, and no predisease antibody levels, the last of which means we cannot rule out the possibility of antibody sequestration in diseased tissues as an explanation for low circulating antibody levels.

Most of the more detailed studies of antibody functionality in the elderly have been conducted in the context of vaccination. Vaccination is an obvious strategy to restore waning natural anti-CPS immunity in the elderly.

VACCINES AGAINST PNEUMOCOCCAL DISEASE: AN OVERVIEW

The pneumococcal polysaccharide vaccine (PPV) was the first licensed vaccine against the pneumococcus; PPV23 is the current 23-valent formulation. PCV has superior immunogenicity and efficacy in children; the most recent formulation is 13-valent PCV13. Childhood vaccination programs generate herd protection by reducing colonization and thus halting transmission at the population level (63). However, serotype replacement has abrogated much of this benefit in many settings (64, 65). Even without significant levels of serotype replacement, vaccine-type disease remains common in older people after childhood vaccination programs are established (66), and residual non-vaccine-type disease will persist as a public health problem (5).

In the United States, the current recommendation for adults >65 years old is vaccination with PCV13 followed by PPV23 (67). In the United Kingdom, PPV23 is recommended for older adults, but the addition of PCV13 was not deemed to be cost-effective, and the use of PPV23 is to be kept under review (68). Recommendations in other Western European countries vary considerably (69).

CURRENT PNEUMOCOCCAL VACCINATION STRATEGIES PROVIDE POOR PROTECTION IN OLDER ADULTS

The discrepancies in national vaccination policies stem from the poor (and disputed) efficacy of these vaccines in older people. A Cochrane review in 2013 concluded that PPV23 effectively prevents pneumococcal bacteremia and meningitis, including in the elderly (70). It has minimal effect at the mucosal level and thus has not been shown to reduce rates of colonization. The Cochrane review found no effect of PPV23 on rates of (nonbacteremic) pneumococcal CAP or all-cause pneumonia, partially because of the substantial heterogeneity of the studies that were included. Nonetheless, some individual studies—including both observational studies and well-conducted randomized controlled trials (RCTs)—have found PPV23 to be efficacious against pneumococcal pneumonia. For example, one double-blind RCT of elderly Japanese nursing home residents (a population expected to have a high incidence of pneumonia and therefore better positioned to detect a vaccine effect) found a 62% relative reduction in the risk of pneumococcal pneumonia and a 39% relative reduction in the risk of all-cause pneumonia with PPV23 (71). When data from this study were pooled with others for the Cochrane meta-analysis, the effect was no longer significant; however, this does not exclude the possibility of a small protective effect of PPV23 against pneumococcal pneumonia, which would be clinically significant in a high-risk population. An important limitation of the Cochrane review is that many of the studies it included were carried out with a general adult population, with limited data available for age-specific subgroup analyses.

An important study of PPV23 in people ≥65 years old has been published since the Cochrane review (72). This study was observational in nature but employed a test-negative design; this reduces several biases and has been found to be similar to RCTs in providing estimates of the effectiveness of seasonal influenza vaccines (73). The study, carried out in Japan, found that the effectiveness of PPV23 was 27.4% against all pneumococcal CAP and 33.5% against CAP caused by the 23 vaccine serotypes (72). Effectiveness against all-cause pneumonia or mortality was not demonstrated. Furthermore, it was notable that this effect was only statistically significant for persons who had been vaccinated within the previous 2 years.

Conjugated vaccines, while covering fewer serotypes, protect children and young adults against colonization (74, 75). In addition to efficacy against vaccine-type bacteremia and meningitis, PCV13 has been shown to reduce rates of vaccine-type CAP in a single large RCT of older adults (CAPiTA) (76). However, with an efficacy of 45.6%, this vaccine did not show complete protection against vaccine-type disease. PCV13 efficacy declined with increasing age. In a *post hoc* analysis, the overall vaccine efficacy against vaccine-type CAP was 65% in 65-year-old persons but only 40% in 75-year-old persons (77). Furthermore, a concomitant increase in non-vaccine-type disease was noted, resulting in no effect against pneumococcal pneumonia in general, and the all-cause mortality rate was unaffected (76).

PNEUMOCOCCAL VACCINES ARE IMMUNOGENIC IN OLDER PEOPLE

In a study of 74 elderly persons, dialysis patients, and transplant recipients (i.e., without young healthy controls), PPV23 was found to improve anti-CPS IgG levels against three selected vaccine serotypes (6, 14, and 23) and not only to improve opsonic activity but to strengthen the correlation between IgG levels and opsonic activity, suggesting that vaccine-induced antibodies are more potent than naturally acquired antibodies (78). A study of 219 adults ≥70 years old found that PCV7 was more immunogenic (as measured by the concentration and function of postvaccine anti-CPS IgG) than PPV23 for all but one of the PCV7 serotypes (79). However, a larger study ($n = 599$) of adults 50 to 80 years old found that PCV7 and PPV23 were equally immunogenic (as defined by IgG concentrations) at 1 month and 1 year following vaccination (58). No functional tests were performed. The reason for the discrepant results of these two studies remains unclear. A randomized study of nursing home residents ≥80 years old found that both PPV23 and PCV7 were immunogenic in this

population, with the conjugate vaccine resulting in higher IgG levels and opsonic activity for some serotypes and both vaccines equally immunogenic for others (80). The effects of single-dose versus boosted vaccination, in various combinations, have been assessed in a number of studies but with conflicting results (reviewed in reference 81).

The immune responses to PPV23 across an elderly population are heterogeneous. One study has suggested that a 4-fold increase in the IgG concentration from the baseline following vaccination is protective against recurrent pneumococcal CAP in the elderly (82). That study had a number of limitations (including low rates of confirmed pneumococcal etiology in cases of CAP) and has not been replicated.

The differential effects of the two vaccines on B cells have been studied extensively. In a cohort of 348 persons 50 to 70 years old, the antibody responses were similar to those in previous studies. PCV7 led to greater anti-CPS IgG concentrations than PPV23 for some, but not all, serotypes—four out of seven in this case (83). However, serotype-specific memory B cell concentrations increased for all seven serotypes following PCV7 but decreased following PPV23 (84). This is consistent with the T-dependent immunogenicity of PCV7. Importantly, repeated doses of unconjugated polysaccharide vaccines do not result in immune boosting—rather, the antibody response is inferior to that following primary vaccination (hyporesponsiveness) (85). Memory B cell depletion has been implicated in this phenomenon (84), which can be avoided by spacing vaccine administrations by at least 5 years (86). It is unclear whether repeated natural exposure to pneumococcal antigens is associated with hyporesponsiveness, but this intriguing hypothesis has been proposed as an additional mechanism of pneumococcal immunodeficiency in the elderly (84) and is an important topic for future research.

The above studies based all analyses on blood samples taken up to 1 month postvaccination. Another study randomized 252 persons 50 to 80 years old to vaccination with either single-dose PPV23 or PCV7 or PCV boosted with either PPV23 or repeat PCV7 and monitored them for 2 years (87). Surprisingly, there was no significant difference in the quantity of circulating serotype-specific memory B cells at 2 years among the four groups. Two-year levels of serotype-specific memory and plasma cells were closely correlated with baseline serotype-specific IgG levels and not with the IgG levels at 7 or 28 days postvaccination. The authors concluded that preexisting natural antipneumococcal immunity was a more important driver of the postvaccine immune response than the type or schedule of vaccine administered. No functional assays were carried out, and there were no young adult controls, but this remains an important study. It is unclear why those authors found no difference in memory B cell concentrations between PPV- and PCV-vaccinated persons while other authors found a dramatic difference (84), but different experimental methodologies and sampling time points in the various studies are possible explanations.

Although some authors have found durable memory B cell responses following either PPV or PCV, clinical and antibody-based studies are less reassuring. PPV-induced antibody levels decline in elderly people over 5 years (86); while they may not decline to the prevaccination baseline, clinical data consistently show reduced protective efficacy over time, suggesting that this decline is relevant and clinically significant (72, 88). Similar declines in opsonic function over time were seen in older adults who received PCV13 (89). The immunological properties of PCV13 (T cell-dependent immunity leading to lasting immunological memory) suggest that any decline in efficacy would be of a lesser magnitude than that of PPV23; however, immunosenescence may well interfere with this. In the CAPiTA trial of PCV13 in people >65 years old conducted over 4 years, clinical efficacy did not appear to decline over time (76), although efficacy was lower in the oldest participants (77). This suggests that there is an age-related component to the clinical protective response following primary vaccination with PCV13. A longer follow-up period is required to determine the duration of protection in the elderly, but conjugate vaccines do appear to confer longer clinical protection than polysaccharide vaccines.

PNEUMOCOCCAL VACCINATION IS MORE IMMUNOGENIC IN YOUNG PEOPLE THAN IN ELDERLY PEOPLE

One study compared the anti-CPS antibody levels in 58 volunteers >65 years old and 44 controls <45 years old 28 days after they had received PPV23 (no prevaccination levels were taken) (90). For the majority of serotypes, antibody levels did not differ significantly between the two groups. However, opsonic antibody titers against all but one serotype (18C) were markedly higher in the younger persons. Antibody potency (opsonization titer divided by the antibody concentration) was at least 2-fold higher for all serotypes in younger persons than in elderly persons, while the amount of antibody needed to achieve a 1:8 opsonization index (a putative protective level) in young persons was less than half of that in the elderly persons. Thus, while uncontrolled studies had shown an improved antipneumococcal immune response following vaccination in elderly people, this is far less impressive than the immune response generated by the same vaccine in healthy young people.

We are unaware of any direct comparison studies of the immunogenicity of PCV in older and younger people. Murine studies have explored this question, but the results were markedly different from those expected in humans on the basis of the current state of knowledge and will therefore not be discussed here (91).

ANTI-CPS IGM RESPONSES ARE MARKEDLY DEFICIENT IN OLDER PEOPLE

In one study, the authors acquired serum samples from 45 healthy elderly persons and 55 healthy young controls, all of whom had been vaccinated 4 weeks previously with PPV23, and tested them against three representative serotypes, 14, 18C, and 23F (92). In keeping with previous studies, the absolute anti-CPS IgG levels were similar in the two groups, but the younger adults had higher opsonic activity and potency than the older persons (albeit not achieving statistical significance for serotype 18C). Young adults commonly demonstrated high levels of opsonic activity even with low antibody levels (i.e., the correlation between antibody levels and opsonic activity was poor), whereas in the elderly, antibody levels and activity were tightly correlated. IgM made a disproportionately significant contribution to opsonic activity; when IgM was removed from the young persons' samples, their opsonic activity was decreased, with a stronger correlation between their IgG levels and opsonic function. When all serum samples were depleted of IgM and reanalyzed, the opsonic activity of the elderly serum samples did not decline and the differences in opsonic activity between old and young persons were no longer statistically significant. The authors concluded that reduced functionality of IgM rather than IgG was responsible for the lower opsonic capacity of elderly persons than younger persons.

The kinetics of IgM could partially explain the above findings; unlike IgG, postvaccination IgM levels rise more slowly, and to a lower peak, in elderly persons than in younger persons (93). All of the samples in the above study were taken quite soon after vaccination. Little is known regarding the duration of IgM responses in the elderly beyond 28 days postvaccination, and thus, the relevance of this laboratory-based study to long-term clinical protection is not certain. However, additional research has shown that the underlying IgM B cell responses to vaccination, in addition to IgM activity itself, are also diminished in the elderly.

A study comparing 14 elderly persons with young controls examined the immune response to two of the PPV23 serotypes (14 and 23F) and found that the serotype 14-specific IgM level did not rise significantly following vaccination in the elderly (though the anti-23F IgM level did) (94). Opsonophagocytic activity in the elderly improved following vaccination, and this was correlated with IgG levels but not with IgM levels and was significantly lower than the opsonophagocytic activity of young vaccine recipients, consistent with previous studies. Flow cytometric analysis showed differences between the postvaccination B cell phenotypes of young and elderly persons; both absolute and relative numbers of $CD27^+$ IgM^+ (IgM memory) B cells were reduced in the elderly. The serotype-specific immune response in the elderly was dominated by switched memory B cells ($CD27^+$ IgM^-). This difference in B cell

populations explained the poor IgM response in the elderly and may provide a key insight into the underlying reasons for poor vaccine-induced clinical protection in this population, but the small numbers of both people and serotypes examined are an important limitation of this study.

Switched memory B cells are part of a T cell-dependent immune response, while IgM memory B cells are T independent (45). Regulatory T cell populations are reduced in the elderly (95); this has been implicated in altered inflammatory responses and susceptibility to pneumonia in the elderly (reviewed in reference 41). Therefore, alterations in T-dependent immunity coupled with a reduction in T-independent IgM memory B cells leaves elderly people vulnerable on two fronts.

IgM defects are unlikely to be the sole reason for the increased susceptibility of elderly people to pneumococcal disease. However, by virtue of its pentameric structure, IgM would be expected to agglutinate and opsonize more efficiently than IgG, and thus, even small defects in IgM levels or function would be expected to have a disproportionate impact. IgM is also key to activating the complement cascade in response to pneumococci (46). While the IgM response to PCV has not been widely studied in the elderly, it is key to the immune response to conjugated vaccines in children (96). Furthermore, PCV-induced IgM antibodies appear to confer cross-protection against some nonvaccine serotypes in children (97)—this has not been demonstrated in the elderly but could represent another domain in which IgM is of key importance. For now, the above data must be regarded as hypothesis generating rather than conclusive, but they are intriguing nonetheless.

ANTIBODIES HAVE MUCOSAL, AS WELL AS SYSTEMIC, ACTIVITY

It is generally reported that IgM and IgA are the principal antibodies present at mucosal surfaces (98, 99), although the relative contributions of different globulin fractions to total antibody levels vary markedly among different organ systems (100). IgA-mediated defense against pneumococci is limited, as all pneumococci synthesize an efficient IgA1 protease, abrogating its protective effect (48). In the final part of this review, we will briefly explore the nature of mucosal antipneumococcal immunity and its relationship with age.

There is a degree of overlap between the mucosal and systemic humoral immune systems, and each is capable of influencing the other (99). Antigens from the nasal mucosal surface are presented to nasopharynx-associated lymphoid tissue (NALT), leading to both local and systemic immune responses. Germinal centers in NALT are responsible for generating B cells that secrete IgA and IgM at the mucosal surface. Furthermore, systemic antibodies can be transported from blood to mucosal surfaces.

SYSTEMIC EXPOSURE TO PNEUMOCOCCAL ANTIGENS VIA VACCINATION CAN LEAD TO MUCOSAL PROTECTION

One study found that PPV leads to an increase in the levels of all classes of anti-CPS antibody in secretions (specifically, saliva and tears; nasal secretions were not studied) (101). Notably, the increases in salivary IgG (4.5-fold) and IgM (4.0-fold) were more pronounced than that of IgA (2.0-fold). However, the functional and clinical effects of these antibodies have not been explored.

In young adults, systemic immunization with PCV13 leads to high concentrations of antipneumococcal IgG in serum, which spills over into the nasal mucosal compartment and can, by virtue of its agglutinating properties, prevent the development of pneumococcal colonization (49). This is likely to be the mechanism for the reduction of pneumococcal colonization following infant vaccination.

MUCOSAL EXPOSURE TO PNEUMOCOCCAL ANTIGENS CAN GENERATE BOTH SYSTEMIC AND LOCAL RESPONSES

As outlined earlier, the upper respiratory mucosa represents humans' first point of contact with the pneumococcus. Transient pneumococcal exposure (in a human challenge model where persons were inoculated but did not become colonized) resulted in

the generation of mucosal antiprotein antibodies but not anti-CPS antibodies and no change in systemic antibody levels (102). Prolonged exposure via colonization leads to increases in functional local and systemic anti-CPS antibodies (36).

Without vaccination, antipneumococcal antibody levels at respiratory mucosal surfaces are too low to prevent colonization. However, "priming" by experimental pneumococcal colonization is protective against subsequent colonization up to 1 year later (36)—whether this is due specifically to mucosal antibodies, serum antibodies (à la vaccination), T cell immunity, or a combination of these remains undetermined.

In addition to inducing mucosal and systemic antipneumococcal antibodies, human pneumococcal colonization leads to an increase in the number of pneumococcus-specific memory $CD4^+$ IL-$17A^+$ T cells (Th-17 cells) (103). When stimulated by pneumococci *in vitro*, IL-17A secreted by these Th-17 cells enhanced the phagocytic killing of pneumococci by alveolar macrophages. Importantly, this Th-17 increase is seen both in peripheral blood and in the lung itself, thus providing evidence of traffic of acquired immune memory from the upper to the lower respiratory tract. However, an alternative hypothesis is that microaspiration of pneumococci during colonization directly induces local T cell infiltration and differentiation within the lungs.

In summary, pneumococci are capable of stimulating a specific immune response at the mucosal surface in addition to generating systemic immunity. The multifaceted mucosal immune response includes both specific antibodies and memory T cells, and a response in the upper respiratory tract may be echoed in the lower respiratory tract. High concentrations of anti-CPS antibodies at the nasopharyngeal surface can prevent pneumococcal acquisition. A mucosal vaccine against pneumococci could be a promising strategy to provide protection for the vulnerable elderly population.

MUCOSAL ANTIPNEUMOCOCCAL IMMUNITY IS AFFECTED BY AGING

Detailed studies of mucosal immunosenescence in general have only been undertaken with mice; it appears that nasal immune function deteriorates with age, but at a rate similar to that of systemic immunity, whereas intestinal mucosal immunity "ages" at a higher rate (104). Murine studies have demonstrated impaired innate antipneumococcal nasal mucosal immunity with increasing age, primarily stemming from macrophage dysfunction (105). Nasal antibodies have not been studied in elderly humans, but salivary antipneumococcal antibodies have been shown to decrease in both concentration and rate of secretion with age (106). We are currently recruiting a cohort of older adults who will undergo experimental human pneumococcal inoculation (ISRCTN ID 10948363) in order to inform our understanding of colonization dynamics, natural antibody generation, and nasopharyngeal mucosal immune responses in this population.

MURINE STUDIES OF ADJUVANTED MUCOSAL PNEUMOCOCCAL VACCINES HAVE SHOWN PROMISE

Studies of strategies of mucosal vaccination against pneumococci have only been undertaken in murine models (reviewed in reference 107) and examined both protein antigens and CPS. The most intriguing findings from these studies have been the effect of novel adjuvants on restoring the immune response in aged mice to both protein and polysaccharide antigens. Addition of CpG oligodeoxynucleotides (CpG-ODN) was found to improve the systemic and mucosal antibody responses to conjugated pneumococcal serotype 9V CPS administered nasally to young mice (108). CpG-ODN enhances antibody production through stimulation of type 1 helper T cells; the underlying mechanism of this remains uncertain (109). This same adjuvant restored the antibody response of aged mice to conjugated serotype 14 CPS administered systemically (110). For nasally administered pneumococcal surface protein A (PspA), a dual-adjuvant strategy of CpG-ODN and a plasmid expressing Flt3 ligand was required to induce similar antibody levels (serum and mucosal IgG and IgA) in young and old mice (111). This strategy also enhanced PspA-specific $CD4^+$ T cell responses in old mice and protected these mice against nasopharyngeal colonization.

It must be emphasized that mouse IgA, having a configuration different from that of human IgA, is less susceptible to cleavage by pneumococcal IgA protease. Thus, if the above findings are to be applicable to human vaccination, it will be essential to demonstrate either that antibodies are a dispensable component of the mucosal immune response or that other immunoglobulins—such as secretory IgM and IgG—are sufficient for protection of humans. If the relative dysfunction of anti-CPS IgM in elderly humans is indeed of clinical significance, then this may prove to be the Achilles' heel of this vaccination strategy, unless an adjuvant can be identified that can restore the function of IgM in the elderly. With this caveat in mind, an appropriately adjuvanted mucosal vaccine could still have enormous potential for reducing the burden of pneumococcal disease in the elderly.

ALTERNATIVE ANTIBODY TARGETS

This review has focused on anti-CPS antibodies. These antibodies are induced by natural exposure to pneumococci and are also the antigens employed in all currently licensed pneumococcal vaccines. Furthermore, there is a substantial body of literature comparing anti-CPS immunity in young and elderly adults. However, the pneumococcus also expresses a variety of surface proteins that are conserved across different serotypes, many of which have been proposed as vaccine candidates (112) and indeed have been explored in mucosal vaccines as outlined above. Antiprotein immune responses have been demonstrated following colonization (36) and may contribute to naturally acquired protection against colonization (34), although their mechanistic significance has not been definitively established (113). In children, studies are conflicting regarding whether antiprotein antibodies confer protection or serve as a marker of exposure and increased risk of disease (114, 115). Antiprotein antibody levels are reduced in the elderly (42). Antiprotein antibody levels rise following pneumococcal disease in older adults (116), and there is a suggestion that their functionality may not be adversely affected by aging, though these findings remain preliminary (E. German et al., unpublished data). Apart from these and the above-mentioned murine studies of mucosal antiprotein immunity, we are unaware of any substantial body of work exploring the nature of aging and antiprotein immunity, and this topic must be prioritized in future research.

CONCLUSION

Impaired naturally acquired CPS immunity leaves elderly people vulnerable to pneumococcal disease. The same factors responsible for this reduction in naturally acquired immunity also result in suboptimal functional antibody responses to current pneumococcal vaccines. PCV13 has overcome some, but by no means all, of the immunological limitations of PPV23. Reduced antibody functionality combined with limited serotype coverage means that pneumococcal vaccination in the elderly does not deliver as substantial a benefit as would be expected.

If anti-CPS antibodies are to remain the mediator of protection, then improvements in the functionality of aged antibodies—particularly IgM—will need to be induced. A mucosal vaccine, with an appropriate adjuvant, would be an attractive strategy. Vaccination strategies seeking to exploit noncapsular antigens or T cell-mediated immunity have shown a degree of promise in early-phase studies of young adults but have yet to achieve their full potential (117). Careful studies of antiprotein immunity in the elderly would guide the exploration of such a vaccination strategy for older adults. Future studies should investigate the dynamics of colonization and mechanisms of naturally acquired immunity in the elderly in greater detail, as well as explore the nature of respiratory mucosal immunity in the elderly to better inform vaccine development for this growing and vulnerable population.

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Hugh Adler studied medicine at University College Dublin (Ireland) and undertook postgraduate training at St. Vincent's University Hospital and the Mater Misericordiae University Hospital (Dublin), specializing in general internal medicine. He became a member of the Royal College of Physicians Ireland in 2013 and completed a Diploma in Tropical Medicine and Hygiene at the Liverpool School of Tropical Medicine (LSTM) in 2014. Following this, he spent 6 months at King Edward VIII University Hospital (Durban, South Africa) as a visiting researcher in pediatric HIV. This experience sparked his interest in global health and in infections in the immunocompromised. Hugh has been a clinical research fellow in the Department of Clinical Sciences at LSTM since 2015. As part of his Ph.D., he is establishing a controlled human *S. pneumoniae* infection model in cohorts of increasing age and exploring the immune responses to pneumococcal colonization in this population.

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