CVI Accepted Manuscript Posted Online 19 April 2017
Clin. Vaccine Immunol. doi:10.1128/CVI.00004-17
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1 **Pneumococcal capsular polysaccharide immunity in the elderly**

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9 Running head: Pneumococcal immunity in the elderly

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Abstract

Immunity against pneumococcal infections is impaired in older people, and current vaccines are poorly protective against pneumococcal disease in this population. Naturally-acquired immunity against 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 over 65 years of age. However, the benefits of polysaccharide and protein-conjugated vaccines in this population are small, due to 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.

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Introduction

Streptococcus pneumoniae, or the pneumococcus, is a major cause of morbidity and mortality in the elderly. People aged over 65 experience up to a five-fold increase in the incidence and mortality of pneumococcal community-acquired pneumonia (CAP) relative to those aged under 65 (1, 2). In the United States, an estimated 600,000 episodes of pneumococcal CAP occur annually, with a total cost to society of US$4.85bn (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 in the elderly than any other age-group (7, 8). Pneumococcal bacteremia is associated with substantial mortality whether in isolation or when associated with confirmed organ infection, and is associated with increased incidence and mortality 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 (CPS) antibodies are a recognized correlate of protection and are common to both the natural and vaccine-induced responses against 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. 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

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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 1 lists examples of studies that attempted to define the rate of pneumococcal colonization in elderly subjects (defined as either >60 or >65 years 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, polymerase chain reaction (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 in 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, 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—4.2%) (15-17), the addition of oral swabs and the combination of traditional culture and PCR can estimate rates of colonization (if defined as ≥1 sample from any site

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testing positive by any method) to as high as 23% in an elderly population (20), or 34% if saliva is also sampled (21).

Thus, while classical microbiological analysis on nasopharyngeal samples from elderly subjects 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 will define colonization as the isolation of pneumococci from the nasopharynx by culture-based methods.

Pneumococcal colonization and naturally-acquired anti-pneumococcal 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, 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 anti-pneumococcal immunity.

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Throughout childhood, adolescence and early adulthood, immunity against pneumococcus improves with age. Children aged under two years have high rates (over 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, along with a corresponding reduction in pneumococcal disease (28). It seems that repeated colonization episodes lead to the development of protective immunity against 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: non-specific anti-pneumococcal 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 non-specific 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 anti-pneumococcal immunity in older adults is more akin to that of young adults than to that of infants.

Young adults experience very low 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 aged under one year) (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 (see TABLE 1), they are at extremely high risk of pneumococcal disease.

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

135 *Naturally-acquired pneumococcal CPS antibodies: an overview*

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

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

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

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

152 *Antibodies are a key product of nasopharyngeal colonization and protect against disease*

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

167 *Clearance of colonization is a complex process*

168 Antibodies have an important role in the protection against becoming colonized. In mice, passive
169 transfer of antibodies lead to agglutination of bacteria following intranasal challenge, which causes
170 the bacteria to clump and become more vulnerable to mucociliary clearance (48). Pneumococcal
171 antibody-mediated agglutination has also been demonstrated in humans following vaccination with
172 pneumococcal conjugate vaccine (PCV) (49). In this study, naturally-acquired antibodies were
173 present in the nasopharynx prior to vaccination, but not in sufficient levels to induce agglutination.

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Murine studies have suggested that the clearance of established colonization is primarily mediated by CD4 cells and interleukin 17 (IL-17), with a possible contribution from anti-protein 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 Figure 1.

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 reacquisition of pneumococcus, 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 in order 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 in neutrophil function with age (54); however, in the interests of 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

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and plasma cell differentiation. Pneumococcal polysaccharide vaccination of the elderly volunteers led to some improvement in IgM levels and IgM memory B cell percentages, but not to the same degree as in younger subjects. 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 (56)). This is an emerging field, and there is a dearth of human studies relevant to this topic outside of 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 when compared with 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 for IgM. The authors noted that serotype-specific IgM concentrations and opsonic activity were poorly correlated, unlike those of IgG. When the decline in antibody level and function are combined, this strongly suggests that antibody defects are responsible for (or at least contribute towards) the age-related increase in vulnerability to pneumococcus.

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Impaired opsonic functionality relative to antibody levels is seen in immunosuppression secondary to a wide variety of etiologies. Although not directly comparable to 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 of HIV-uninfected subjects, 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. Sera from patients in the acute and convalescent stages of various types of pneumococcal disease were compared with age-matched controls (62). Only 27% of subjects with pneumococcal disease had IgG to their infecting serotype at time of presentation (compared to 37% of controls and 42% of colonized subjects). Furthermore, acute antibodies from infected subjects had significantly lower opsonic activity than those of controls or colonized subjects and were less protective via passive transfer in a lethal murine challenge model (20% survival vs 100%). Sixty-two percent of convalescent sera had detectable IgG following pneumococcal disease, which demonstrated good function in >50% of patients. Important limitations of this study include substantial loss to follow-up between the acute and convalescent phases, no reporting of ages, and no pre-disease 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 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 denotes the current 23-valent formulation. The pneumococcal protein-conjugated vaccine (PCV) has superior immunogenicity and efficacy in children; the most recent

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formulation is the 13-valent PCV13. Childhood vaccination programs generate herd protection by reducing colonization and thus halting transmission at a 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 USA, current recommendations for adults aged over 65 years advise vaccination with PCV13 followed by PPV23 (67). In the UK, PPV23 is recommended in 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 (non-bacteremic) pneumococcal CAP or all-cause pneumonia, partially due to the substantial heterogeneity of 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 in 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 risk reduction of pneumococcal pneumonia, and a 39% relative risk reduction of all-cause pneumonia with PPV23 (71). When data from this study was 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 against pneumococcal pneumonia

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from PPV23, which would be clinically significant in a high-risk population. An important limitation of the Cochrane review is that the many of the studies it included were carried out in a general adult population, with limited data available for age-specific subgroup analyses.

An important study of PPV23 in people aged ≥ 65 years 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 vaccine effectiveness for 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 was not demonstrated against all-cause pneumonia or mortality. Furthermore, it was notable that this effect was only statistically significant for subjects who had been vaccinated within the previous two years.

Conjugated vaccines, while covering fewer serotypes, protect against colonization in children and young adults (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 in older adults (CAPiTA) (76). However, with vaccine 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, overall vaccine efficacy against vaccine-type CAP was 65% in 65-year-old subjects but only 40% in 75-year-olds (77). Furthermore, a concomitant increase in non-vaccine type disease was noted, resulting in no effect against pneumococcal pneumonia in general, and all-cause mortality was unaffected (76).

Pneumococcal vaccines are immunogenic in older people

In a study of 74 elderly subjects, 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

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than naturally acquired antibodies (78). A study of 219 adults aged ≥70 years found that PCV7 was more immunogenic (as measured by concentration and function of post-vaccine anti-CPS IgG) than PPV23 for all but one of the PCV7 serotypes (79). However, a larger study (n = 599) of adults aged 50—80 years found that PCV7 and PPV23 were equally immunogenic (as defined by IgG concentrations) at one month and one year following vaccination (58). No functional tests were performed. The reasons for the discrepant results between these two studies remains unclear. A randomized study of nursing home residents aged ≥80 years 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 (81)).

The immune responses to PPV23 across an elderly population are heterogeneous. One study has suggested that a four-fold increase in IgG concentration from baseline following vaccination is protective against recurrent pneumococcal CAP in the elderly (82). This 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 subjects aged 50—70 years, the antibody responses were similar to previous studies: PCV7 lead 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

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least five 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 one month post-vaccination. Another study randomized 252 subjects aged 50—80 years to vaccination with either single-dose PPV23 or PCV7, or PCV boosted with either PPV23 or repeat PCV7, and followed them for two years (87). Surprisingly, there was no significant difference in the quantity of circulating serotype-specific memory B cells at two years between 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 from 7 or 28 days post-vaccination. The authors concluded that pre-existing natural anti-pneumococcal immunity was a more important driver of the post-vaccine immune response than the type or schedule of vaccine administered. No functional assays were carried out, and there were no young adult control subjects, but this remains an important study. It is unclear why these authors found no difference in memory B cell concentrations between PPV and PCV-vaccinated subjects while other authors found a dramatic difference (84), but different experimental methodologies and sampling timepoints between 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 five years (86); while they may not decline to the pre-vaccination 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.

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In the CAPiTA trial of PCV13 in over-65s, conducted over four years, clinical efficacy did not appear to decline over time (76), although efficacy was lower in the oldest participants (77). This suggests that there an age-related component to the clinical protective response following primary vaccination with PCV13. A longer period of follow-up would be 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 anti-CPS antibody levels in 58 volunteers aged >65 years and 44 controls aged <45 years, 28 days after they had received PPV23 (no pre-vaccination levels were taken) (90). For the majority of serotypes, antibody levels did not differ significantly between the two groups. However, opsonic titers against all but one serotype (18C) were markedly higher in the younger subjects. Antibody potency (opsonization titer divided by the antibody concentration) was at least two-fold higher for all serotypes in younger subjects than in elderly subjects, while the amount of antibody needed to achieve a 1:8 opsonization index (a putative protective level) in young subjects was less than half of that in the elderly subjects. 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 with what would be expected in human subjects based on the state of current 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 sera from 45 healthy elderly subjects and 55 healthy young controls, all of whom had been vaccinated four weeks previously with PPV23, and tested them

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against three representative serotypes: 14, 18C and 23F (92). In keeping with previous studies, absolute anti-CPS IgG levels were similar between both groups, but the younger adults had higher opsonic activity and potency than the older subjects (albeit not achieving statistical significance for serotype 18C). Young adults commonly demonstrated high levels of opsonic activity even with low levels of antibody (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 subjects' samples, their opsonic activity was decreased, with 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 sera did not decline and the differences in opsonic activity between old and young subjects were no longer statistically significant. The authors concluded that reduced functionality of IgM rather than IgG was responsible for the reduced opsonic capacity of elderly subjects when compared with younger subjects.

The kinetics of IgM could partially explain the above findings: unlike IgG, post-vaccination IgM levels rise more slowly, and to a lower peak, in elderly subjects compared with younger subjects (93). All 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 post-vaccination, 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 fourteen elderly subjects with young controls examined the immune response against two of the PPV23 serotypes (14 and 23F) and found that serotype 14-specific IgM did not rise significantly following vaccination in the elderly (though anti-23F IgM did) (94). Opsonic activity improved following vaccination in the elderly, and this was correlated with IgG levels but not with IgM levels, and was significantly lower than the OPA of young vaccine recipients, consistent with

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previous studies. Flow cytometric analysis showed differences between young and elderly subjects in their post-vaccination B cell phenotypes: 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 subjects and serotypes examined) are an important limitation of this study.

Switched memory B cells comprise 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 (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 pneumococcus (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 non-vaccine 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

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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 varies markedly between different organ systems (100). IgA-mediated defense against pneumococcus 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 anti-pneumococcal 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 nasopharyngeal-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 levels of all classes of anti-CPS in secretions (specifically saliva and tears; nasal secretions were not studied) (101). Notably, the fold 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 serum concentrations of anti-pneumococcal IgG, 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 in pneumococcal colonization following infant vaccination.

Mucosal exposure to pneumococcal antigens can generate both systemic and local responses

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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 subjects were inoculated but did not become colonized) resulted in the generation of mucosal anti-protein 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 one 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 pneumococcal-specific memory $CD4^+$ $IL\text{-}17A^+$ 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 in both 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 a 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 pneumococcus could be a promising strategy to provide protection for the vulnerable elderly population.

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Mucosal anti-pneumococcal immunity is affected by aging

Detailed studies of mucosal immunosenescence in general have only been undertaken in mice: it appears that nasal immune function deteriorates with age, but at a similar rate to systemic immunity, whereas intestinal immunity mucosal "ages" at a faster 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 mucosal vaccination strategies against pneumococcus have only been undertaken in murine models (reviewed in (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 response 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 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 was protective against nasopharyngeal colonization in these mice.

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

501 *Alternative antibody targets*

502 This review has focused on anti-CPS antibodies. These antibodies are induced by natural exposure
503 to pneumococcus and are also the antigens employed in all currently-licensed pneumococcal
504 vaccines. Furthermore, there is a substantial body of literature comparing anti-CPS immunity in
505 young and elderly adults. However, the pneumococcus also expresses a variety of surface proteins
506 which are conserved across different serotypes, many of which have been proposed as vaccine
507 candidates (112) and indeed have been explored in mucosal vaccines as outlined above. Anti-
508 protein immune responses have been demonstrated following colonization (36) and may contribute
509 to naturally-acquired protection against colonization (34) although their mechanistic significance has
510 not been definitively established (113). In children, studies are conflicting regarding whether anti-
511 protein antibodies confer protection or serve as a marker of exposure and increased risk of disease
512 (114, 115). Anti-protein antibody levels are reduced in the elderly (42). Anti-protein antibody
513 levels rise following pneumococcal disease in older adults (116), and there is a suggestion that their
514 functionality may not be adversely affected by aging, though these findings remain preliminary
515 (German E et al, unpublished data). Apart from these, and the above-mentioned murine studies of

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516 mucosal anti-protein immunity, we are unaware of any substantial body of work exploring the
517 nature of aging and anti-protein immunity, and this topic must be prioritized in future research.

518 *Conclusion*

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

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

535 **Acknowledgments**

536 No specific funding was awarded for this MiniReview article, but the authors' research programs are
537 funded by the UK Medical Research Council (Grant MR/M011/569/1) and the Bill & Melinda Gates
538 Foundation (Grant OPP111728). In addition, the authors gratefully acknowledge Dr Emma Smith

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and Dr India Wheeler for their assistance with the literature review, and Patrick Lane for assistance with the figures.

Figure 1

Anti-capsular antibodies can be acquired naturally (following pneumococcal exposure, e.g. colonization, or through pneumococcal disease) or via vaccination. They facilitate pneumococcal killing via opsonisation. 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.

Figure 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 capsular polysaccharide (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.

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Author biographies

Dr Hugh Adler

Hugh Adler studied medicine in University College Dublin (Ireland) and undertook postgraduate training in St Vincent's University Hospital and the Mater Misericordiae University Hospital (Dublin), specialising 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 six months in 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 in LSTM since 2015. As part of his PhD, he is establishing a controlled human infection model of *Streptococcus pneumoniae* in cohorts of increasing age and exploring the immune responses to pneumococcal colonisation in this population.

Dr Daniela M Ferreira

Daniela Ferreira has a BSc in Biological Sciences and a PhD in Immunology from the University of São Paulo (Brazil). She trained at Butantan Institute (São Paulo) for 9 years on vaccine development, novel adjuvants and immunization routes with a special focus on mucosal vaccination. In 2008 Daniela received the Robert Austrian Research Award in Pneumococcal Vaccinology for her work in this field. After a spell at the University of Leicester as a Research Fellow, Daniela joined LSTM in December 2009 and was appointed to Senior Lecturer within the Department of Clinical Sciences in 2015. To accelerate vaccine research, her team has developed a unique experimental human pneumococcal carriage model. The key areas of her research are 1) nasal and lung immune responses 2) formulation, development and testing novel pneumococcal vaccines, and 3) the effect of influenza virus co-infection on pneumococcal carriage.

Prof Stephen B Gordon

Stephen Gordon was educated at the University of Cambridge and trained in General Medicine in Oxford, Zambia and Belfast. He specialised in Respiratory Medicine in Sheffield (Clinical Lecturer) and Malawi (2 Wellcome Trust Fellowships). He joined LSTM in 2005, with a remit to establish laboratory and clinical research on susceptibility to pulmonary infections. Stephen's research in Sheffield and Malawi focused on susceptibility to respiratory infection, particularly on the effect of HIV infection on susceptibility to pneumococcal disease. The work demonstrated that pulmonary mucosal defence was regulated differently than systemic defence against infection, and could be

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perturbed by environmental exposures including indoor air pollution. Since 2015 he has been resident in Blantyre, Malawi as the Director of the Malawi-Liverpool-Wellcome Trust (MLW) Clinical Research Programme. The MLW Programme has a mission to benefit human health, particularly in sub-Saharan Africa, through excellent translational science focused on infectious disease in hospital and the community.

Dr Jamie Rylance

Jamie Rylance is a clinical academic, specialising in General Internal Medicine and Respiratory Medicine. He has a strong interest in health in low income countries, having worked as a doctor in Tanzania and Malawi. His clinical research has focussed on the intersection of chronic respiratory disease and acute respiratory infection, and its treatment in resource limited settings. His laboratory work has sought explanations for propensity to pneumonia, examining mucosal immunity and redox balance in the lung in the context of household air pollution generated by the domestic use of biomass fuels. He is now senior clinical lecturer in LSTM and leads the clinical implementation of the controlled human infection model of *Streptococcus pneumoniae*.

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Table 1: Examples of studies attempting to define the rate of pneumococcal colonization in older people by culture-based and/or molecular methods

First author (reference)	Year	Country	Number sampled	Age (years)	Site sampled	Analysis	Rate of detection of pneumococci, n (%)
Becker-Dreps (15)	2015	USA	210	81.4 (6.3)*	NP	Classical microbiology	**4 (1.9%)**
Almeida (16)	2014	Portugal	3,361	74.56 (8.2)*	NP	Classical microbiology with multiplex PCR confirmation of culture-positive specimens	61 (1.8%)
					OP		15 (0.4%)
					Overall		**76 (2.3%)**
Flamaing (17)	2012	Belgium	503	80.3 (10.0)*	NP	Classical microbiology (a subset were also tested with *lytA* PCR—see published paper for full details)	**21 (4.2%)**
Esposito (18)	2016	Italy	417	73.97 (6.66)*	OP	PCR	**41 (9.8%)**
Ansaldi (19)	2013	Italy	283	NR	NP	Culture-enriched PCR	**53 (18.7%)**
Van Deursen (20)	2016	Netherlands	330	72.7 (68.7—79.0)†	NP	Classical microbiology	16 (5%)
						PCR	32 (10%)
					OP	Classical microbiology	16 (5%)
						PCR	58 (18%)
					Overall		**75 (23%)**
Krone (21)	2015	Netherlands	270**	69 (NR)*	NP	Culture-enriched PCR	13 (5%)
					OP		31 (11%)
					Saliva		76 (28%)
					Overall		**91 (34%)**

NP: Nasopharyngeal; NR: Not reported; OP: Oropharyngeal; PCR: Polymerase chain reaction

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Table 1: Examples of studies attempting to define the rate of pneumococcal colonization in older people by culture-based and/or molecular methods
* Mean (SD)
† Median (IQR)
**135 subjects, sampled both pre and post influenza-like illness. At a participant level, 65/135 (48%) tested positive on at least one occasion.

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Table 2: Summary of clinical and laboratory measurements of anti-pneumococcal immunity in young and old adults

	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 titres	Robust	Declines with age
Circulating natural anti-CPS antibody opsonophagocytic activity	Robust	Declines profoundly with age
Anti-CPS antibody titres following vaccination	Robust	Robust
Anti-CPS antibody opsonophagocytic activity following vaccination	Robust	Declines with age
Memory B cell responses 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 non-bacteremic pneumococcal pneumonia	Probable	Possible
Clinical efficacy of PPV against pneumococcal bacteremia/meningitis	Undisputed	Undisputed
Clinical efficacy of PCV against non-bacteremic 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

CPS, capsular polysaccharide; PCV, pneumococcal conjugate vaccine; PPV, pneumococcal polysaccharide vaccine.