CVI Journals.ASM.org

Streptococcus pneumoniae Serotype 1 Burden in the African Meningitis Belt: Exploration of Functionality in Specific Antibodies

S. Blumental,[a,b] J. C. Moïsi,[c] L. Roalfe,[b] M. Zancolli,[b] M. Johnson,[b] P. Burbidge,[b] R. Borrow,[d] S. Yaro,[e] J. E. Mueller,[f] B. D. Gessner,[c] D. Goldblatt[b]

Paediatric Infectious Diseases Unit, Hôpital Universitaire des Enfants Reine Fabiola, Brussels, Belgium[a]; UCL Institute of Child Health, London, United Kingdom[b]; Agence de Médecine Préventive, Paris and Ferney-Voltaire, France[c]; Public Health England Vaccine Evaluation Unit, Manchester, United Kingdom[d]; Centre Muraz, Bobo-Dioulasso, Burkina Faso[e]; EHESP School of Public Health, Paris, France[f]

***Streptococcus pneumoniae* serotype 1 (Sp1) constitutes an important cause of seasonal endemic meningitis in all age groups in the African meningitis belt. Despite a higher meningitis incidence, the Burkinabé population has an Sp1-specific antibody seroprevalence similar to that reported in the United Kingdom (UK). We aimed to establish whether the opsonophagocytic activity (OPA) of pneumococcal IgG naturally present in Burkina Faso differs from that seen in individuals in the UK and to compare the OPAs generated by natural and vaccine-induced immunity. Samples collected from pneumococcal vaccine-naive Burkinabé and UK subjects were matched for age (1 to 39 years) and anti-Sp1 IgG level, analyzed for OPA to 3 *S. pneumoniae* serotypes (1, 5, and 19A), and compared to postvaccine samples. Furthermore, the Burkinabé samples were assessed for IgG avidity and serotype-specific IgM concentrations. One hundred sixty-nine matched serum samples from both populations were selected. A greater proportion of Burkinabé subjects aged 1 to 19 years had functional Sp1 activity (OPA ≥ 8) compared to UK subjects (12% versus 2%, $P < 0.001$); however, the proportions were similar among adults (9%). The correlation between Sp1 IgG concentration and OPA was good ($P < 0.001$), but many individuals had nonfunctional IgG, which was not related to avidity. While the Sp1 IgM concentrations correlated with OPA, not all of the function in serum samples with low IgG could be attributed to IgM. Finally, vaccine-induced Sp1-specific IgG was more functional than equivalent amounts of naturally occurring IgG. In conclusion, despite a substantially higher pneumococcal meningitis incidence, no decreased functional immunity to Sp1 could be evidenced in the Burkinabé population compared to that in the population from the UK. Furthermore, the naturally induced antibodies were less functional than vaccine-induced antibodies.**

Streptococcus pneumoniae is a major pathogen responsible for 14.5 million annual infections worldwide and >800,000 deaths in children <5 years of age (1). In addition to being an important commensal of the human nasopharynx, this bacterium is frequently involved in respiratory tract infections (e.g., acute otitis media, sinusitis, and pneumonia) or invasive diseases, like septicemia and meningitis. Following the introduction of effective *Haemophilus influenzae* type b vaccines, *S. pneumoniae* emerged worldwide as the leading cause of bacterial meningitis in the youngest age group, with a majority of cases occurring in developing countries (1). In industrialized countries, infants, the elderly, and immunocompromised patients constitute the main risk groups for pneumococcal meningitis, while it remains relatively rare in older children and healthy adults (2, 3). In contrast, in the African meningitis belt (sub-Saharan Africa), most cases and the majority of deaths occur in children >5 years of age and working-age adults. The incidence in this age group is approximately 10 cases per 100,000, which is significantly higher than the 0.3 to 0.6/100,000 recorded in developed countries (4). Annually, people living in this region experience *S. pneumoniae* meningitis hyperendemicity that follows a defined seasonal pattern (as observed for *Neisseria meningitidis*) and is associated with a historical case-fatality ratio of ≥50% among hospitalized persons (5, 6). Recently published data estimate that serotype 1 (Sp1) accounts for a large majority of the recorded *S. pneumoniae* meningitis episodes among persons >5 years old (5–8).

With the licensing of pneumococcal conjugate vaccines (PCV), invasive pneumococcal disease, including meningitis, decreased significantly in those countries in which PCV was introduced into their national immunization programs (9). The first licensed vaccine (the 7-valent PCV [PCV7]) contained the 7 serotypes that most frequently caused invasive pneumococcal disease (IPD) in developed countries, and it did not include serotype 1. In 2009, 10- and 13-valent conjugates were licensed, which included serotypes 1 and 5, both of which are important in developing countries, such as those in the African meningitis belt. While many African countries have recently introduced PCV10 and PCV13 with help from Gavi, The Vaccine Alliance's advanced market commitment (10), data evaluating their impact are not yet available. Furthermore, due to the unique features of pneumococcal meningitis in the meningitis belt, including the predominance of

Received 12 December 2014 **Returned for modification** 17 January 2015
Accepted 28 January 2015

Accepted manuscript posted online 4 February 2015

Citation Blumental S, Moïsi JC, Roalfe L, Zancolli M, Johnson M, Burbidge P, Borrow R, Yaro S, Mueller JE, Gessner BD, Goldblatt D. 2015. *Streptococcus pneumoniae* serotype 1 burden in the African meningitis belt: exploration of functionality in specific antibodies. Clin Vaccine Immunol 22:404–412. doi:10.1128/CVI.00758-14.

Editor: H. F. Staats

Address correspondence to S. Blumental, sophie.blumental@ulb.ac.be, or D. Goldblatt, d.goldblatt@ucl.ac.uk.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/CVI.00758-14.

doi:10.1128/CVI.00758-14

one pneumococcal serotype with a strong seasonal pattern and a high incidence persisting throughout the whole adult life, it is not clear what impact infant immunization with serotype 1-containing conjugates will have on the overall incidence of pneumococcal meningitis in this region.

To date, the exact reasons underlying the pattern of infection and the importance of Sp1 in sub-Saharan Africa remain poorly understood. While climatic factors may predispose the meningitis belt population to meningitis (as for *N. meningitidis*), it is unclear why they should favor Sp1 rather than predispose the population in general to all pneumococcal serotypes. Similarly, malnutrition and HIV infection should not preferentially predispose to serotype 1 disease, and these factors are not higher in the meningitis belt than in other African countries. To investigate whether the absence of natural immunity may explain Sp1 meningitis in this region, we first conducted a cross-sectional serosurvey among healthy persons 1 to 39 years of age in Bobo-Dioulasso, Burkina Faso (11). We observed an age-associated increase in pneumococcal IgG seroprevalence similar to that seen among the United Kingdom (UK) unvaccinated population (12). No serological difference could explain the differences in the age-specific meningitis incidence rates. However, the determination of IgG level by enzyme-linked immunosorbent assay (ELISA) alone has been demonstrated to be insufficient for properly reflecting protection against pneumococcus, especially in unvaccinated individuals (13–15). Naturally occurring pneumococcal serotype-specific IgG may not be as functional as vaccine-induced antibodies. A lack of functionality in these antibodies might explain an enhanced susceptibility of individuals to invasive pneumococcal disease and contribute to the particular features of *S. pneumoniae* meningitis that were previously described.

We therefore explored whether the naturally occurring Sp1-specific antibodies identified in Burkinabé subjects are functionally equivalent to those in the UK population. We were also able to compare the natural levels of functional antibodies to functional antibodies measured following the administration of conjugate or polysaccharide vaccines in infants and adults, respectively, in the United Kingdom (16, 17).

MATERIALS AND METHODS

Study design. (i) Specimen collection. This study was part of a large cross-sectional serological survey that was conducted from March to April 2006 by the Agence de Médecine Préventive (AMP) and Centre Muraz among healthy persons 1 to 39 years old in Bobo-Dioulasso, Burkina Faso. Detailed materials and methods are published elsewhere (11). In summary, at the peak of a meningococcal meningitis epidemic, serum samples were obtained from 622 healthy subjects from various age categories and were subsequently assayed for several parameters, including serotype-specific pneumococcal IgG. Clinical and demographic data were recorded in parallel, and *S. pneumoniae* carriage was assessed using nasopharyngeal swabs, as described previously (11). Following the collection and processing of blood specimens, the serum samples were transported to and stored at −80°C at the Public Health England (PHE) Meningococcal Reference Laboratory, Manchester, United Kingdom.

(ii) Selection of the study population. *(a) Burkinabé and UK unvaccinated cohorts.* A random subset of 169 serum samples collected from healthy pneumococcal vaccine-naive Burkinabé subjects were chosen in each category, defined by age group and Sp1-specific IgG concentration stratum, for analysis in this study. Of a total 169 samples, the number of subjects within each category ranged from 7 to 18 (see the supplemental material). One hundred sixty-nine age- and Sp1 IgG-matched serum samples from pneumococcal vaccine-unimmunized UK subjects collected during a national epidemiological survey between 2000 and 2004 were selected to serve as the comparison group (12).

(b) S. pneumoniae-vaccinated cohorts. To compare the levels of natural immunity to those induced after vaccination with a serotype 1-containing vaccine, we used samples previously collected in clinical trials. We utilized samples obtained from infants during a study of PCV13 coordinated in the United Kingdom by the National Vaccine Evaluation Consortium (17). Fifty-eight serum samples collected at 5 months of age from infants receiving PCV13 (Prevnar 13; Pfizer Ltd., Kent, United Kingdom) at 2 and 4 months of age were selected within specific categories of Sp1 IgG levels, as follows: 10, 24, and 24 subjects in categories with Sp1 IgG levels of <0.35, 0.35 to 1, and >1 μg/ml, respectively.

Adult samples collected during a study of a 23-valent pneumococcal polysaccharide vaccine (PPV) were also available for comparison (16). The volunteers were age 50 to 80 years and had received the PPV 6 months before sampling. A subset of 58 samples was randomly chosen to be analyzed in our study, as no Sp1 IgG results were available.

Laboratory assays. (i) IgG serum concentrations. For Burkinabé and UK naive cohorts, the serotype-specific IgG concentrations for multiple serotypes (1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F, plus 3, 7F, and 19A for Burkina Faso only) were previously determined at the PHE (Manchester, United Kingdom) using a multiplex bead-based assay, as described elsewhere (12, 18), which included the adsorption of sera with serotype 22F polysaccharide and cell wall polysaccharide (CPS). The results of the IgG levels in these two populations have been published elsewhere (11, 12). The sera collected from the vaccinated cohorts were separated, aliquoted, stored at −80°C, and tested for specific antibodies to Sp1, Sp5, and Sp19A using the WHO reference ELISA after adsorption with CPS and 22F polysaccharide (19). These assays were carried out at the Institute of Child Health, University College London, London, United Kingdom.

(ii) Determination of opsonophagocytic activity. All serum samples were investigated for functional activity against serotypes 1, 5, and 19A using an opsonophagocytic killing assay in a multiplex fashion, as described previously (20, 21). The opsonophagocytic killing assay values were expressed as the inverse of the antibody titer required to kill 50% of a standard inoculum of bacteria. The results were expressed as the geometric mean titer (GMT) along with the 95% confidence interval (CI) and as the proportion of subjects with an opsonophagocytic activity (OPA) of ≥ 8.

(iii) Avidity testing. Burkinabé and PPV-vaccinated adult samples were further assessed for IgG avidity. Avidity for antibodies to serotypes 1 and 19A was measured by modifying an assay developed for anti-*H. influenzae* type b (Hib) avidity by incorporating an ammonium thiocyanate elution step into the pneumococcal ELISA (22). Antibody avidity was expressed as an avidity index corresponding to the molar concentration of ammonium thiocyanate required to produce a 50% reduction in absorbance. The avidity index results are presented as the geometric mean concentration (GMC) with the 95% CI.

(iv) IgM detection. Serum samples from the Burkinabé cohort were assayed for serotype-specific IgM antibodies to serotypes 1, 5, and 19A using an ELISA based on the WHO reference IgG ELISA (19). IgM plates were incubated with alkaline phosphatase-conjugated goat anti-human IgM (μ-chain specific) antibody (Sigma, St. Louis, MO, USA) at a 1:1,000 dilution in antibody buffer for 2 h at room temperature. Antibodies were detected following incubation with substrate solution, and the optical density (OD) at 405 nm was read.

Statistical analysis. Assuming a 15% difference in the proportion of UK versus Burkinabé sera with a serotype 1 OPA of ≥ 8, a sample size of 186 per group was originally targeted to distinguish between Burkinabé and UK OPA findings with 80% power and a type I error of 0.05. After the primary results analysis, the final sample size was reduced to 169 in each unvaccinated group.

Statistical analysis was performed using the GraphPad Prism 4.0 software (GraphPad, Inc., San Diego, CA, USA). A *t* test and Mann-Whitney U test were used to compare continuous parametric and nonparametric

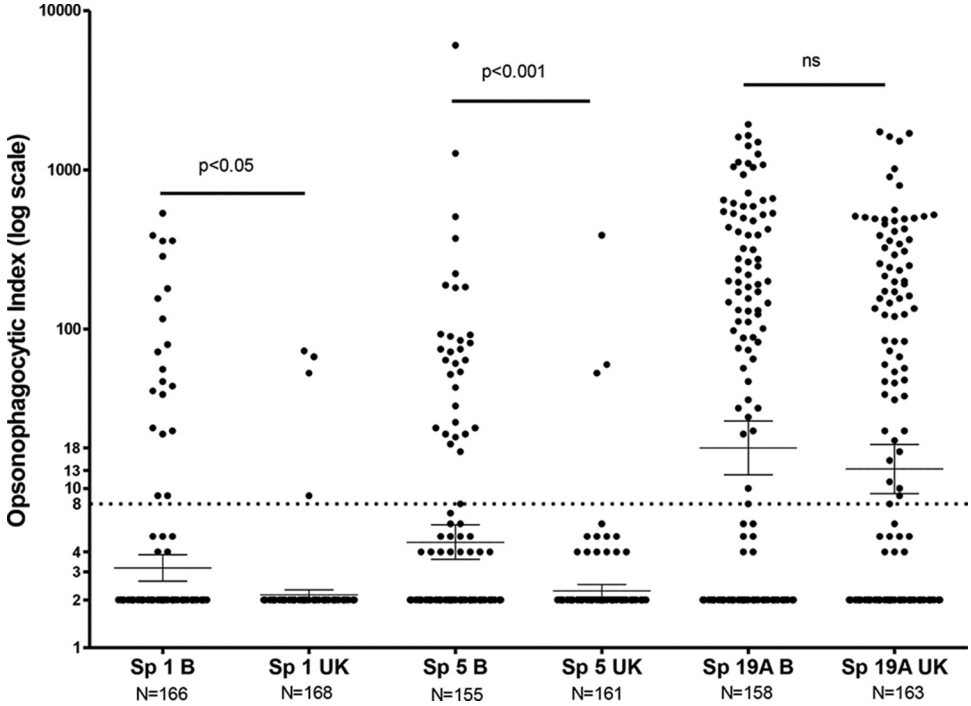

FIG 1 Comparison of OPA titers against 3 *S. pneumoniae* serotypes between the Burkina Faso cohort and UK controls matched for age and IgG level. The dotted line indicates OPA positivity cutoff (≥8); the thin horizontal lines indicate GMC with the 95% confidence interval. Sp, serotype; B, Burkina Faso; UK, United Kingdom; N, number of subjects; ns, nonsignificant.

variables, respectively, chi-square or Fisher's exact tests were used to assess the differences in proportions, and Spearman's correlation analysis was used to test for associations between different metrics. The Kruskal-Wallis test, followed by Dunn's posttests, were used for multiple comparisons of the continuous variables. Moreover, linear regression models (endpoint, log OPA) and logistic regression models (endpoint, OPA ≥ 8) were developed to investigate the impact on opsonophagocytic activity of several variables alone or in association. The assessed variables were age, serotype-specific antibody concentrations (IgG and IgM for Sp1), and epidemiological covariates of sex, history of meningitis or contact with a meningitis case, pneumococcal colonization status at the time of serum collection, smoking, household crowding (defined as >4 people sharing the same room), meningitis vaccination history, and reported antibiotic use within the month before sampling. Models were developed for each of the 3 studied serotypes using the Stata Software 12.1.

For each analysis, a two-tailed *P* value of <0.05 was considered statistically significant.

Ethics. This study and the first part of the surveillance project mentioned above (11) were both approved by the Comité d'Ethique de la Recherche en Santé at Centre Muraz, Ouagadougou, Burkina Faso, and were supported by the Ministry of Health of Burkina Faso. For each Burkinabé individual, written informed consent was obtained before inclusion in the study. The use of sera collected either in the United Kingdom by the Health Protection Agency (HPA) or during clinical trials run by the HPA is governed by permission granted by the Eastern Multi Center Research Ethics Committee (MREC). The pediatric PCV13 study was approved by the UK Medicines and Healthcare Products Regulatory Authority and the Leicestershire, Northamptonshire and Rutland Research Ethics Committee 1 (EudraCT no. 2010-023865-22) (17). The study involving PPV-vaccinated adult volunteers was conducted in Hertfordshire and Gloucestershire, United Kingdom, and was approved by the Public Health Laboratory Service and local ethics committees (16).

RESULTS

OPA in natural immunity. (i) Selected study cohorts. After selection, we included 169 samples in each of the study groups from Burkina Faso and the UK; 100 samples were from children (40 between 1 and 4 years and 60 between 5 and 14 years), and 69 were from adolescents/adults (35 between 15 and 19 years and 34 between 20 and 29 years) (see the supplemental material). Within each cohort, the 3 main categories of Sp1 IgG levels (<0.35 μg/ml, 0.35 to 1 μg/ml, and >1 μg/ml) were almost equally represented. Out of both cohorts, a small subset of serum samples (from 1 to 14, depending on the serotype) had to be excluded from the OPA analysis for technical reasons.

While two-thirds of the Burkina Faso and UK samples contained Sp1 IgG levels of ≥0.35 μg/ml (the putative protective level), Sp1 OPAs were low in both cohorts, but the GMTs were significantly higher in the Burkina Faso than the UK group (GMT [95% CI], 3.1 [2.6 to 3.8] and 2.1 [2 to 2.3]) ($P < 0.05$) and the proportions of subjects with an OPA of ≥8 were 12% and 2% among the Burkina Faso and UK groups, respectively ($P < 0.001$) (Fig. 1). Stratification by age group confirmed that among the subjects 1 to 19 years old, a greater proportion of Burkinabé individuals had functional Sp1 activity than did the UK subjects (13% versus 1%, respectively; $P < 0.001$), whereas the proportions were similar among adults (9% for both groups).

Functional activity against Sp5 followed a similar pattern of low OPA titers (Fig. 1). The proportions of subjects with OPA of ≥8 were higher in the Burkinabé cohort for all age groups, including adults (Burkina Faso versus UK, 17% versus 0.8% and 38% versus 7% in the <20-year and 20- to 39-year age groups, respectively [$P < 0.001$ and $P < 0.01$, respectively]). The Sp5-specific

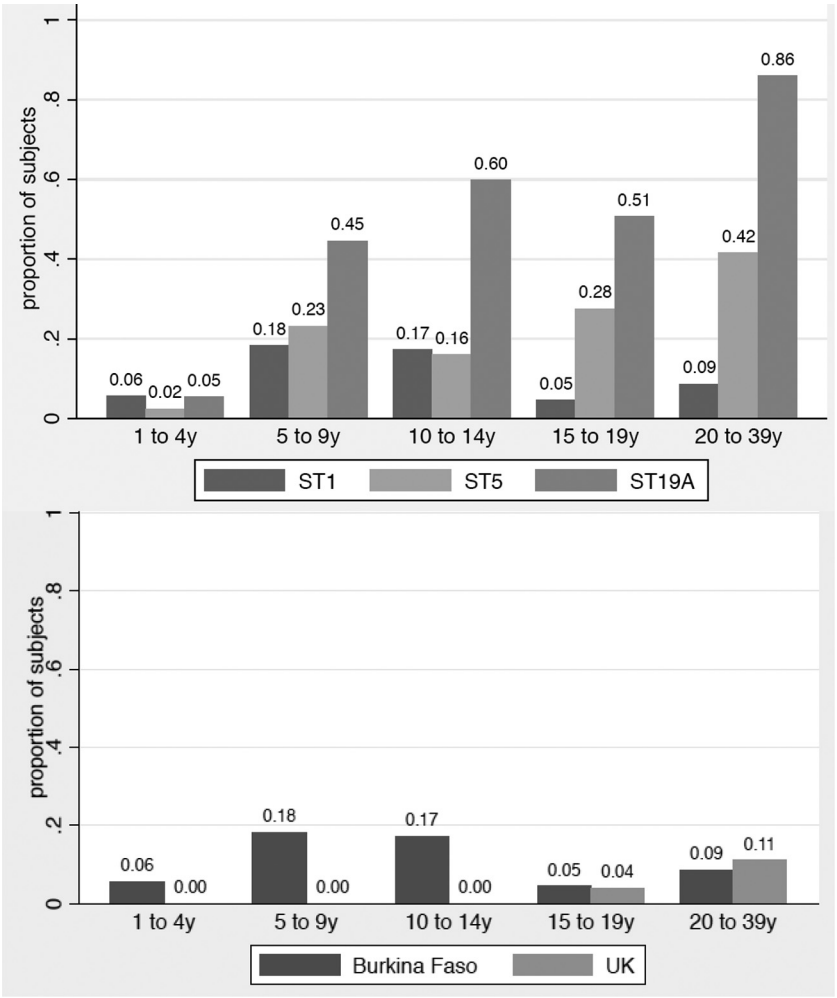

FIG 2 (A) Proportion of Burkinabé subjects with functional antibodies (OPA ≥ 8) by serotype (ST) and age group (y, years) after standardization for age and IgG level. (B) Proportion of Burkinabé and UK subjects with Sp1 functional antibodies (OPA ≥ 8) by age group after standardization for age and IgG level.

IgG levels were, however, higher in the UK than in the Burkina Faso group (Sp5 IgG GMT [95% CI], 0.57 μg/ml [0.49 to 0.66 μg/ml] and 0.36 μg/ml [0.28 to 0.48 μg/ml], respectively; $P < 0.01$).

The Burkina Faso and UK groups had similar functional activities against Sp19A (Fig. 1). The proportion of individuals with functional activity against Sp19A was low among children age ≤4 years (5% and 12% in the Burkina Faso and UK groups, respectively) and increased from age 5 years up to 41 to 63% (Burkina) and 42 to 85% (UK) during adulthood.

(ii) Extrapolation to the population level. To minimize sampling bias and extrapolate our data to the general population level, a sampling weight was attributed to each sample category based on the official demographic data of the two countries (source, http://esa.un.org/unpd/wpp/Excel-Data/population.htm) and the Sp1 IgG distributions observed in previous studies (11, 12) (see the supplemental material). After correction, the functional activity patterns observed in the Burkina Faso cohort remained similar, with very low OPAs for Sp1 and Sp5 (Fig. 2A). Moreover, the proportion of subjects with an OPA of ≥8 increased with age for Sp5 and Sp19A, whereas for Sp1, the maximum was reached in school-aged children and adolescents, with a subsequent decline. A comparison with weighted UK data confirmed much higher Sp1 functional activity observed during childhood among the Burkinabé population but similar levels during adolescence and adulthood (Fig. 2B).

Factors influencing OPA value. (i) Correlation between OPA and natural antibodies. Within the Burkina Faso cohort, the correlation between IgG concentration and OPA was significant for the three serotypes 1, 5, and 19A (Spearman test, $P < 0.001$ each) (Fig. 3). A similar correlation was not observed in the UK naive population due to the low numbers of individuals with functional activity for the two serotypes for which IgG values were available (Sp1 and Sp5 only). In linear regression models with the serum samples from the Burkinabé subjects, serotype-specific IgG titers fitted OPA values for Sp5 and Sp19A better than for Sp1 (R^2 = 0.57, 0.50, and 0.17, respectively; P values, <0.0001, <0.0001, and 0.002, respectively).

Interestingly, for Sp1, the specific IgM level could be assessed on the whole cohort and was also positively associated with OPA values (Fig. 4), with an even stronger impact than IgG, as shown in a multivariate regression model that included both covariates ($R^2 = 0.22$; slope β, 0.51 and 0.2; P values, 0.019 and 0.04 for IgM

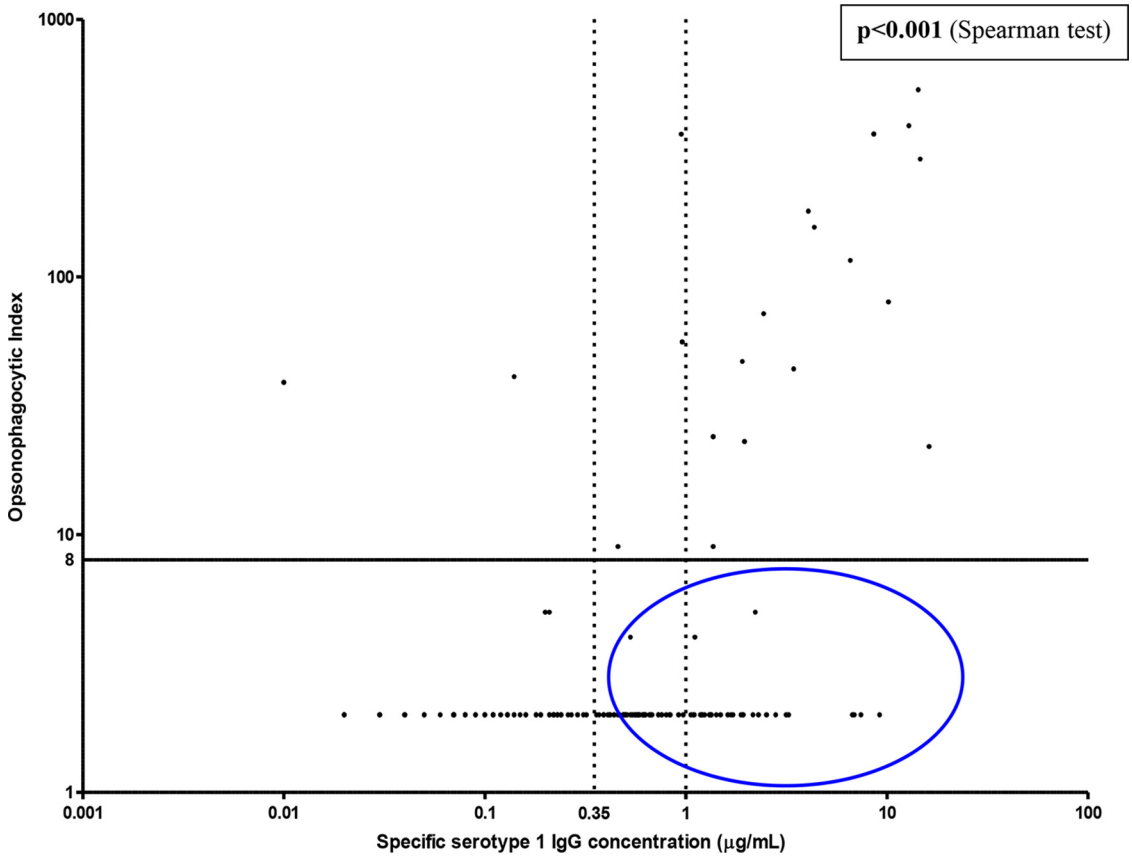

FIG 3 Correlation between OPA titers and IgG concentrations for Sp1 in the Burkina Faso cohort ($n = 166$ serum samples). Similar results were obtained for the two other serotypes. The bold horizontal line indicates the OPA positivity cutoff (≥8); the dotted lines indicate the two IgG putative protection levels (0.35 and 1 μg/ml). The blue circle indicates discrepant results with nonfunctional IgG.

and IgG, respectively). As expected, a significant association was found between Sp1 IgG and IgM titers, but R^2 was low ($R^2 = 0.1$, $P < 0.001$).

(ii) Epidemiological factors. In contrast to serotype-specific IgG concentrations, the influence of age alone on OPA was not statistically significant in any of the models. Several epidemiological variables available for Burkinabé subjects (see Materials and Methods) were also included in a multivariate linear regression model, but none of them led to a significant association with OPA values (regardless of the serotype). The lone exception was an association between household crowding and higher Sp1 OPA levels (slope β = 0.42, $P = 0.024$).

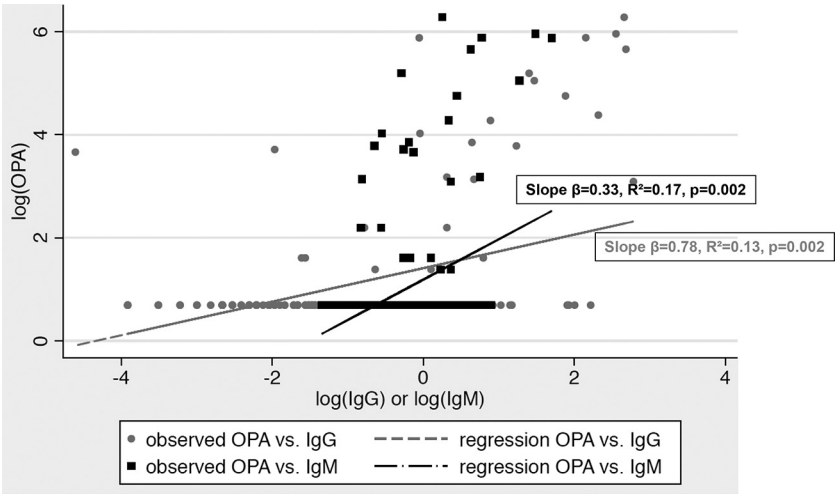

FIG 4 Association between OPA values and specific IgG/IgM titers for Sp1 within the Burkina Faso cohort (univariate linear regression model, log data).

Assessment of OPA/IgG discrepant results. Despite a good correlation between IgG and OPA titers, some discrepant results were observed for the three serotypes; these consisted either of nonfunctional IgG (defined as an IgG level of ≥0.35 μg/ml and an OPA of <8 [Fig. 3, lower right quadrant, see blue circle]) or a positive OPA despite an IgG level of <0.35 μg/ml (Fig. 3, upper left quadrant).

(i) Avidity testing. To establish whether low avidity might explain the presence of nonfunctional Sp1-specific IgG, avidity was measured on 65 selected Burkina Faso samples. No difference in IgG avidity by killing function was demonstrated (avidity index geometric mean [95% CI], 359 [213 to 604] and 218 [95 to 500] in Sp1 nonkilling and killing groups, $n = 46$ and 17 people, respectively; $P = 0.29$). Moreover, IgG avidity was significantly higher for Sp19A than for Sp1 (avidity index geometric mean [95% CI], 748 [511 to 1,090] and 327 [214 to 500], $n = 58$ and 65 people, respectively; $P = 0.007$). This difference in avidity between the specific antibodies of the two serotypes remained significant even after stratification for individuals with and without killing function (OPA, ≥8 or <8, respectively).

(ii) Logistic regression. A multivariate logistic regression model revealed that for Sp1, neither the majority of epidemiological covariates (listed in Materials and Methods) nor IgM values were associated with the presence of nonfunctional IgG (defined as an IgG level of ≥0.35 and an OPA of <8). The exceptions included female sex and age >5 years, which were associated with increased odds of having nonfunctional antibodies (odds ratio [OR] [95% CI], 2.1 [1 to 4.2] and 3.5 [1.6 to 7.7], respectively; $P = 0.04$ and 0.002, respectively), while prior contact with a meningitis case was associated with decreased odds (OR [95% CI], 0.3 [0.14 to 0.78]; $P = 0.01$).

(iii) High OPA with low IgG. For the three studied serotypes, outlier samples showing functional activity despite low IgG levels were further investigated for serotype-specific IgM titers (Burkina Faso cohort) and compared to controls matched for OPA values but having higher IgG concentrations (>1 μg/ml) (Fig. 3, upper left quadrant). No difference in IgM GMTs was found (Table 1). However, the analysis was limited by the small number of samples involved.

Comparison between natural and vaccine-induced immunity. A random subset of 31 IgG-matched samples from each of the pneumococcal vaccine-naive or vaccinated (PCV13 infants/PPV adults) cohorts were compared for their Sp1-specific OPAs. The analysis was first restricted to UK unimmunized and immunized populations to avoid confounders. As there were small numbers of positives in the UK unvaccinated group, the analysis was extended to the Burkinabé vaccine-naive population. All samples from the four groups contained Sp1 IgG levels of >0.35 μg/ml and were matched between the groups according to their Sp1 IgG level. Infants and adults who had received Sp1-containing vaccines had statistically higher OPA GMTs than those of individuals with naturally induced Sp1 antibody activity from either the Burkinabé or the UK unimmunized cohorts (Kruskal-Wallis test, $P < 0.0001$; and Dunn's multiple-comparison posttests) (Fig. 5). However, Sp1-specific antibody avidity remained similar between a subset of unimmunized Burkinabé subjects and a group of PPV-vaccinated adults (avidity index geometric mean [95% CI], 327 [214 to 500] and 206 [168 to 253], $n = 65$ and 54 people, respectively; $P = 0.05$).

Distribution of Sp1 IgM according to age groups. The distribution of Sp1 IgM in the Burkinabé cohort showed lower titers among the youngest children (≤4 years), followed by an increase to a plateau level from ≥5 years of age. This difference remained highly significant even after correction for the IgG sampling bias based upon the general population data (weighted GMTs [μg/ml] [95% CI], 0.54 [0.46 to 0.63] among the 1- to 4-year-old population versus 0.99 [0.79 to 1.26], 0.97 [0.77 to 1.23], 0.93 [0.76 to 1.16], and 0.97 [0.73 to 1.28] among the 5- to 9-, 10- to 14-, 15- to 19-, and 20- to 39-year-old populations; $P < 0.0001$ each).

TABLE 1 Comparison of serotype-specific IgM concentrations among individuals with positive opsonophagocytic activity (≥8), stratified by IgG level[a]

Serotype	Measurement[b]	Serotype-specific IgM level (μg/ml) in group with:		*P*
		Low IgG (<1 μg/ml)	High IgG (>1 μg/ml)	
1	GM (μg/ml)	0.9912	0.9478	>0.05
	Lower 95% CI of GM	0.2882	0.4467	
	Upper 95% CI of GM	3.409	2.011	
	No. of values	5	5	
5	GM (μg/ml)	4.71	3.13	NA[c]
	Lower 95% CI of GM	1.291	1.7	
	Upper 95% CI of GM	17.19	5.76	
	No. of values	3	3	
19A	GM (μg/ml)	2.01	2.055	>0.05
	Lower 95% CI of GM	1.589	1.564	
	Upper 95% CI of GM	2.541	2.7	
	No. of values	28	28	

[a] Individuals were matched for serotype-specific OPA values between the two groups.
[b] GM, geometric mean; CI, confidence interval.
[c] NA, not applicable.

DISCUSSION

In this study, we set out to characterize natural immunity to pneumococcal serotypes in a population residing in the African meningitis belt with sophisticated assays not previously applied to such populations. Our findings provide insights into natural immunity at a population-based level. We found that a high seasonal incidence of serotype 1 pneumococcal meningitis occurs in the African meningitis belt in the presence of a low level of functional immunity that is nevertheless equal to or higher than that observed in a UK resident cohort with much lower disease incidence.

Showing a strong seasonal pattern, a high incidence persisting throughout the whole adult life, and the vast predominance of a single serotype (Sp1) after age 5 years as the causal pathogen, *S. pneumoniae* meningitis assumes specific features in the African meningitis belt. The exact reasons underlying the particular pattern of this infection remain poorly understood. While multiple factors might be responsible for such a pattern, we decided to investigate aspects of host immunity with a focus on humoral immunity, since opsonophagocytosis by antibodies represents a primary mechanism of protection against IPD (23). In an earlier report on this same cohort, the age distribution of serotype-specific pneumococcal IgG in Burkina Faso was found to be comparable to that in a UK-based population, with a high proportion of adult individuals having serotype-specific antibodies above the putative protection level (>60% for Sp1) (11). Despite this high

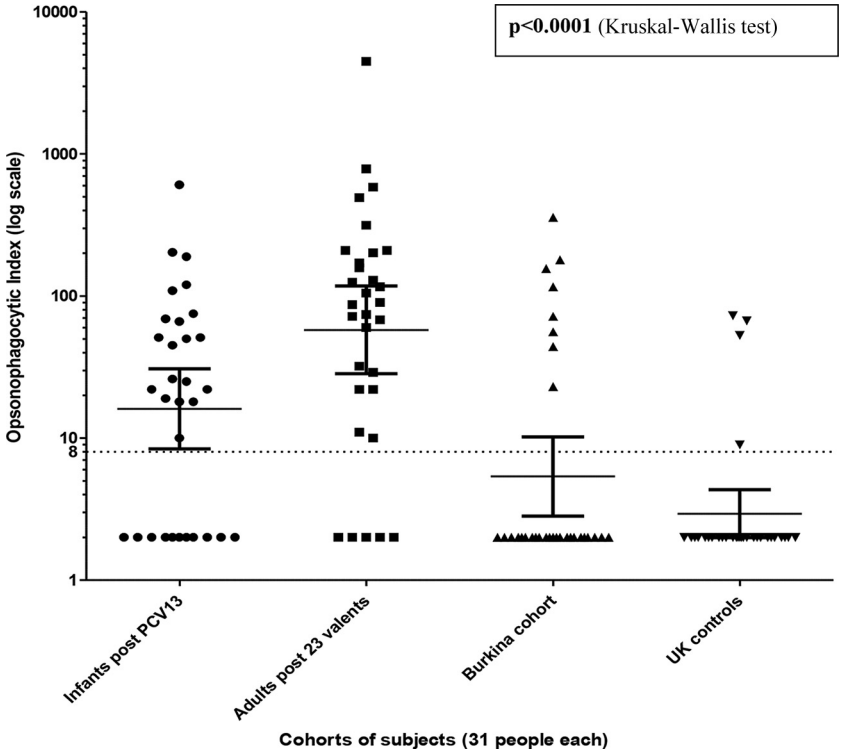

FIG 5 Comparison of Sp1-specific OPA titers between pneumococcal vaccine-naive and vaccinated populations. The dotted line indicates the OPA positivity cutoff (≥8); the solid horizontal bars indicate the GMC with the 95% confidence interval.

seroprevalence of Sp1-specific IgG, we have shown that opsonophagocytic activity is generally low in both populations studied, with <10% of adults and 13% of infants having an OPA of ≥8. Antibody function was similar for adults from the two countries; however, the Burkinabé infants had a higher proportion with positive OPA than did the UK infants (13% versus 1%, respectively). Mueller and colleagues (7) recently reported high rates of Sp1 meningitis in Burkina Faso in the face of relatively little carriage (i.e., a high case-to-carrier ratio), suggesting that the higher functional activity among Burkinabé children might be attributed to higher transmission and more contact with Sp1, leading to periodic boosting of functional antibodies. This hypothesis is supported by our finding of an association between household crowding and functional antibodies in Burkinabé subjects. The low level of functional antibodies in both populations is consistent with the relatively infrequent identification of serotype 1 in carriage in the United Kingdom (24) and in Burkina Faso (7, 11) and the limited opportunities for the development of natural immunity compared to that with other serotypes. It is also possible that serotype-specific factors affect antibody stimulation or the duration of protection. A general defect in humoral immunity among the Burkinabé subjects was ruled out by our data, showing high levels of serotype 19A functional antibodies in this population. Climatic factors (e.g., dry season and Saharan winds) are increasingly recognized for predisposing the meningitis belt population to meningitis, perhaps by altering the nasopharyngeal mucosa, thereby favoring central nervous system invasion by encapsulated bacteria (11, 25, 26). Summarizing the above findings, both UK and Burkinabé populations are susceptible to disease with the relatively invasive serotype 1, due to low natural immunity, but it is only the Burkinabé population that has incidence catalyzed by the presence of a strong environmental risk factor.

Currently, it is uncertain to what extent seroprevalence data reliably predict the protection of naive populations to pneumococcal disease. While over- or underestimations of clinical effectiveness based upon IgG data have been published in vaccine studies (13, 27), some disagreements between clinical observations (carriage or infection) and naturally induced pneumococcal seroprevalence have been reported in large epidemiological surveys. In Burkina Faso, Yaro et al. (11) suggested that all serotypes, regardless of their prevalence in carriage or IPD, had similar patterns of IgG seroprevalence. Similarly, in the United Kingdom, Balmer et al. (12) reported a high seroprevalence of naturally acquired Sp1 and Sp5 IgG, with rapid increases during infancy, despite the rare circulation of those two serotypes in the United Kingdom at the time of the study (4). Both our study and others demonstrate that some antibodies measured by binding assays, particularly naturally occurring antibodies, may be nonfunctional (14, 15, 28). The exact origin of those nonfunctional naturally occurring antibodies remains unclear, but it seems likely that the majority of them arise from cross-reactivity with other nonprotective epitopes belonging to either the same pneumococcal serotype/serogroup or to other enteric (*Escherichia coli* and *Klebsiella* spp.) or nasopharyngeal (*Streptococcus* group B and *H. influenzae* type b) bacterial species, or even to carbohydrates in dietary products, which share identical mono-/polysaccharide determinants with one or more *S. pneumoniae* serotypes (29–32). Our data corroborate the findings from a Finnish study that prospectively demonstrated that children produced poorly functional antibodies against a number of serotypes to which they never were previously exposed (in carriage

or acute otitis media) (14, 33). Since we have now demonstrated that many individuals having naturally induced serotype-specific IgG above the putative protection level (0.35 μg/ml) lack opsonophagocytic function, it suggests that associations between circulating IgG, as measured by ELISA and clinical phenomena (e.g., reduction in incidence of disease), may need to be better understood. The IgG threshold of 0.35 μg/ml, which is considered a correlate of protection for all serotypes following conjugate vaccination (34), does not accurately predict the function of naturally induced serotype 1-specific IgG, although an Sp1 IgG-OPA correlation was found. Recently, an IgG level of 0.71 μg/ml has been proposed as a more accurate correlate of protection for Sp1 after conjugated vaccination (17). Moreover, IgG estimates alone do not take into account the contribution of other immunoglobulin isotypes to opsonophagocytic activity and protection. All of the above emphasizes the utility of using OPA to evaluate natural immunity in unvaccinated populations and the need for further investigation of the relationship between natural antibodies, as measured by OPA and clinical protection.

The reduced functionality of naturally occurring IgG was highlighted by the comparison of OPA values between naive and vaccinated cohorts, as higher opsonophagocytic function was seen postvaccination for subjects with similar IgG concentrations. Interestingly, the avidity of antibodies did not show any difference between immunized and unimmunized subjects. A higher avidity of antibodies has been postulated to contribute to enhanced functionality and to represent a surrogate marker of successful priming of immunological memory after vaccination against *H. influenzae* and *N. meningitidis* (35, 36). However, the literature on the relationship between anti-pneumococcal IgG avidity and OPA is currently conflicting; therefore, the impact of this parameter remains debated (36–38).

In addition to IgG, other antibody isotypes, and in particular IgM, are increasingly thought to participate in protection against pneumococcal infection by facilitating phagocytosis. Due to their pentameric structure, IgM polymers contain 10 antigen-binding sites and are therefore efficient at activating the complement cascade (39). Recent studies have suggested an important contribution of IgM antibodies to the opsonic activity observed after PCV vaccination (40–42). Their contribution persisted for ≥1 year after the last vaccine dose (40) and decreased with age due to a reduced $CD27^+$ B-cell repertoire among elderly people (41). There is little information on the question of protective activity of naturally induced IgM. Our study demonstrated in the vaccine-naive Burkinabé population a significant correlation between IgM and opsonic function that was independent of IgG. However, we failed to show a role for IgM in discrepant IgG/OPA observations in contrast to that seen in vaccinated individuals (40, 42). As our observations might be limited by the small number of subjects, analysis of IgM as a mediator of natural immunity against encapsulated bacteria should be explored further.

ACKNOWLEDGMENTS

This study was supported by two scientific grants from the Belgian Kids' Fund and the Iris Research Network.

We thank all local teams from Centre Muraz and AMP for their active contribution to that work. We also thank all patients and healthy volunteers who agreed to participate in the study.

B.D.G. and J.C.M. work for AMP, which receives grant funding from Pfizer and GSK, manufacturers of pneumococcal conjugate vaccines, as well as Merck, Crucell, Sanofi-Pasteur, and Novartis. R.B. performs contract research on behalf of Public Health England for Baxter Bioscience, GSK, Novartis, Pfizer, Sanofi Pasteur, and Sanofi Pasteur MSD. D.G. has served on ad hoc advisory boards for Pfizer, GSK, and Merck, and the UCL ICH Lab (D.G., L.R., M.Z., M.J., and P.B.) receives contract research funding from Pfizer, GSK, and Merck. S.B. and J.E.M. declare no conflicts of interest.

REFERENCES

1. **O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, Lee E, Mulholland K, Levine OS, Cherian T, Hib and Pneumococcal Global Burden of Disease Study Team.** 2009. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet **374:**893–902. http://dx.doi.org/10.1016/S0140-6736(09)61204-6.
2. **Zangwill KM, Vadheim CM, Vannier AM, Hemenway LS, Greenberg DP, Ward JI.** 1996. Epidemiology of invasive pneumococcal disease in southern California; implications for the design and conduct of a pneumococcal conjugate vaccine efficacy trial. J Infect Dis **174:**752–759. http://dx.doi.org/10.1093/infdis/174.4.752.
3. **Petit G, De Wals P, Law B, Tam T, Erickson LJ, Guay M, Framarin A.** 2003. Epidemiological and economic burden of pneumococcal diseases in Canadian children. Can J Infect Dis **14:**215–220.
4. **Trotter CL, Waight P, Andrews NJ, Slack M, Efstratiou A, George R, Miller E.** 2010. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: England and Wales, 1996–2006. J Infect **60:**200–208. http://dx.doi.org/10.1016/j.jinf.2009.12.008.
5. **Traore Y, Tameklo TA, Njanpop-Lafourcade BM, Lourd M, Yaro S, Niamba D, Drabo A, Mueller JE, Koeck JL, Gessner BD.** 2009. Incidence, seasonality, age distribution, and mortality of pneumococcal meningitis in Burkina Faso and Togo. Clin Infect Dis **48**(Suppl 2):S181–S189. http://dx.doi.org/10.1086/596498.
6. **Leimkugel J, Adams Forgor A, Gagneux S, Pflüger V, Flierl C, Awine E, Naegeli M, Dangy JP, Smith T, Hodgson A, Pluschke G.** 2005. An outbreak of serotype 1 *Streptococcus pneumoniae* meningitis in northern Ghana with features that are characteristic of *Neisseria meningitidis* meningitis epidemics. J Infect Dis **192:**192–199. http://dx.doi.org/10.1086/431151.
7. **Mueller JE, Yaro S, Ouédraogo MS, Levina N, Njanpop-Lafourcade BM, Tall H, Idohou RS, Sanou O, Kroman SS, Drabo A, Nacro B, Millogo A, van der Linden M, Gessner BD.** 2012. Pneumococci in the African meningitis belt: meningitis incidence and carriage prevalence in children and adults. PLoS One **7:**e52464. http://dx.doi.org/10.1371/journal.pone.0052464.
8. **Parent du Châtelet I, Traore Y, Gessner BD, Antignac A, Naccro B, Njanpop-Lafourcade BM, Ouedraogo MS, Tiendrebeogo SR, Varon E, Taha MK.** 2005. Bacterial meningitis in Burkina Faso: surveillance using field-based polymerase chain reaction testing. Clin Infect Dis **40:**17–25. http://dx.doi.org/10.1086/426436.
9. **Black S, Shinefield H, Baxter R, Austrian R, Bracken L, Hansen J, Lewis E, Fireman B.** 2004. Postlicensure surveillance for pneumococcal invasive disease after use of heptavalent pneumococcal conjugate vaccine in northern California Kaiser Permanente. Pediatr Infect Dis J **23:**485–489. http://dx.doi.org/10.1097/01.inf.0000129685.04847.94.
10. **Centers for Disease Control and Prevention (CDC).** 2013. Progress in introduction of pneumococcal conjugate vaccine worldwide, 2000–2012. Morb Mortal Wkly Rep MMWR **62:**308–311.
11. **Yaro S, Njanpop-Lafourcade BM, Drabo A, Idohou RS, Kroman SS, Sanou O, Traoré Y, Sangaré L, Diagbouga SP, Koeck JL, Borrow R, Gessner BD, Mueller JE.** 2014. Antipneumococcal seroprevalence and pneumococcal carriage during a meningococcal epidemic in Burkina Faso, 2006. J Infect Dis **209:**1241–1250. http://dx.doi.org/10.1093/infdis/jit641.
12. **Balmer P, Borrow R, Findlow J, Warrington R, Frankland S, Waight P, George R, Andrews N, Miller E.** 2007. Age-stratified prevalences of pneumococcal-serotype-specific immunoglobulin G in England and their relationship to the serotype-specific incidence of invasive pneumococcal disease prior to the introduction of the pneumococcal 7-valent conjugate vaccine. Clin Vaccine Immunol **14:**1442–1450. http://dx.doi.org/10.1128/CVI.00264-07.
13. **Henckaerts I, Durant N, De Grave D, Schuerman L, Poolman J.** 2007.

Validation of a routine opsonophagocytosis assay to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. Vaccine **25:**2518–2527. http://dx.doi.org/10.1016/j.vaccine.2006.09.029.

14. **Soininen A, Karpala M, Wahlman S, Lehtonen H, Kayhty H.** 2002. Specificities and opsonophagocytic activities of antibodies to pneumococcal capsular polysaccharides in sera of unimmunized young children. Clin Diagn Lab Immunol **9:**1032–1038. http://dx.doi.org/10.1128/CDLI.9.5.1032-1038.2002.
15. **Antilla M, Voutilainen M, Jäntti V, Eskola J, Käyhty H.** 1999. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. Clin Exp Immunol **118:**402–407. http://dx.doi.org/10.1046/j.1365-2249.1999.01077.x.
16. **Goldblatt D, Southern J, Andrews N, Ashton L, Burbidge P, Woodgate S, Pebody R, Miller E.** 2009. The immunogenicity of 7-valent pneumococcal conjugate vaccine versus 23-valent polysaccharide vaccine in adults aged 50–80 years. Clin Infect Dis **49:**1318–1325. http://dx.doi.org/10.1086/606046.
17. **Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, Slack M, Ladhani SN, Miller E, Goldblatt D.** 2014. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis **14**(9)**:**839–846. http://dx.doi.org/10.1016/S1473-3099(14)70822-9.
18. **Lal G, Balmer P, Stanford E, Martin S, Warrington R, Borrow R.** 2005. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine *Streptococcus pneumoniae* serotypes. J Immunol Methods **296:**135–147. http://dx.doi.org/10.1016/j.jim.2004.11.006.
19. **World Health Organization Pneumococcal Serology Reference Laboratories at the Institute of Child Health, University College London, Department of Pathology University of Alabama at Birmingham.** 2013. Training manual for enzyme linked immunosorbent assay for the quantitation of *Streptococcus pneumoniae* serotype specific IgG (Pn PS ELISA). University of Alabama at Birmingham, Birmingham, AL. http://www.vaccine.uab.edu/ELISA%20protocol.pdf.
20. **Burton RL, Nahm MH.** 2006. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. Clin Vaccine Immunol **13:**1004–1009. http://dx.doi.org/10.1128/CVI.00112-06.
21. **Goldblatt D, Southern J, Ashton L, Andrews N, Woodgate S, Burbidge P, Waight P, Miller E.** 2010. Immunogenicity of a reduced schedule of pneumococcal conjugate vaccine in healthy infants and correlates of protection for serotype 6B in the United Kingdom. Pediatr Infect Dis J **29:**401–405. http://dx.doi.org/10.1097/INF.0b013e3181c67f04.
22. **Goldblatt D, Richmond P, Millard E, Thornton C, Miller E.** 1999. The induction of immunologic memory after vaccination with *Haemophilus influenzae* type b conjugate and acellular pertussis-containing diphtheria, tetanus, and pertussis vaccine combination. J Infect Dis **180:**538–541. http://dx.doi.org/10.1086/314901.
23. **Tuomanen EI, Austrian R, Masure HR.** 1995. Pathogenesis of pneumococcal infection. N Engl J Med **332:**1280–1284. http://dx.doi.org/10.1056/NEJM199505113321907.
24. **Flasche S, Van Hoek AJ, Sheasby E, Waight P, Andrews N, Sheppard C, George R, Miller E.** 2011. Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. Plos Med **8:**e1001017. http://dx.doi.org/10.1371/journal.pmed.1001017.
25. **Mueller JE, Gessner BD.** 2010. A hypothetical explanatory model for meningococcal meningitis in the African meningitis belt. Int J Infect Dis **14:**e553–e559. http://dx.doi.org/10.1016/j.ijid.2009.08.013.
26. **Thomson MC, Molesworth AM, Djingarey MH, Yameogo KR, Belanger F, Cuevas LE.** 2006. Potential of environmental models to predict meningitis epidemics in Africa. Trop Med Int Health **11:**781–788. http://dx.doi.org/10.1111/j.1365-3156.2006.01630.x.
27. **Song J, Moseley M, Burton R, Nahm M.** 2013. Pneumococcal vaccine and opsonic pneumococcal antibody. J Infect Chemother **19:**412–425. http://dx.doi.org/10.1007/s10156-013-0601-1.
28. **Soininen A, van den Dobbelsteen G, Oomen L, Kayhty H.** 2000. Are the enzyme immunoassays for antibodies to pneumococcal capsular polysaccharides serotype specific? Clin Diagn Lab Immunol **7:**468–476. http://dx.doi.org/10.1128/CDLI.7.3.468-476.2000.
29. **Crumrine MHFischer GW, Balk MW.** 1979. Immunochemical cross-reactions between type III group B streptococcus and type 14 *Streptococcus pneumoniae*. Infect Immun **25:**960–963.
30. **Lagergård T, Branefors P.** 1983. Nature of cross-reactivity between *Haemophilus influenzae* type a and b and *Streptococcus pneumoniae* types 6A and 6B. Acta Pathol Microbiol Immunol Scand **91:**371–376.
31. **Guirguis N, Schneerson R, Bax A, Egan W, Robbins JB, Shiloach J, Orskov I, Orskov F, Elkholy A.** 1985. *Escherichia coli* K51 and K93 capsular polysaccharides are cross reactive with the group A capsular polysaccharide of *Neisseria meningitidis*: immunochemical, biological, and epidemiological studies. J Exp Med **162:**1837–1851.
32. **Lee CJ, Koizumi K.** 1981. Immunochemical relations between pneumococcal serogroup 19 and *Klebsiella* capsular polysaccharides. J Immunol **127:**1619–1623.
33. **Soininen A, Pursiainen K, Kilpi T, Kayhty H.** 2001. Natural development of antibodies to pneumococcal capsular polysaccharides depends on the serotype: association with pneumococcal carriage and acute otitis media in young children. J Infect Dis **184:**569–576. http://dx.doi.org/10.1086/322794.
34. **World Health Organization.** 2005. Pneumococcal conjugate vaccines: recommendations for the production and the control of pneumococcal conjugate vaccines. WHO Tech Rep Ser **927:**64–98.
35. **Joseph H, Miller E, Dawson M, Andrews N, Feavers I, Borrow R.** 2001. Meningococcal serogroup A avidity indices as a surrogate marker of priming for the induction of immunologic memory after vaccination with a meningococcal A/C conjugate vaccine in infants in the United Kingdom. J Infect Dis **184:**661–662. http://dx.doi.org/10.1086/322790.
36. **Ekström N, Ahman H, Palmu A, Grönholm S, Kilpi T, Käyhty H, FinOM Study Group.** 2013. Concentration and high avidity of pneumococcal antibodies persist at least 4 years after immunization with pneumococcal conjugate vaccine in infancy. Clin Vaccine Immunol **20:**1034–1040. http://dx.doi.org/10.1128/CVI.00039-13.
37. **Anttila M, Voutilainen M, Jäntti V, Eskola J, Käyhty H.** 1999. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. Clin Exp Immunol **118:**402–407. http://dx.doi.org/10.1046/j.1365-2249.1999.01077.x.
38. **Oishi T, Ishiwada N, Matsubara K, Nishi J, Chang B, Tamura K, Akeda Y, Ihara T, Nahm MH, Oishi K, Japanese IPD Study Group.** 2013. Low opsonic activity to the infecting serotype in pediatric patients with invasive pneumococcal disease. Vaccine **31:**845–849. http://dx.doi.org/10.1016/j.vaccine.2012.11.010.
39. **Shyur S, Raff HV, Bohnsack JF, Kelsey DK, Hill HR.** 1992. Comparison of the opsonic and complement triggering activity of human monoclonal IgG1 and IgM antibody against group B streptococci. J Immunol **148:**1879–1884.
40. **Simell B, Nurkka A, Ekström N, Givon-Lavi N, Käyhty H, Dagan R.** 2012. Serum IgM antibodies contribute to high levels of opsonophagocytic activities in toddlers immunized with a single dose of the 9-valent pneumococcal conjugate vaccine. Clin Vaccine Immunol **19:**1618–1623. http://dx.doi.org/10.1128/CVI.00248-12.
41. **Park S, Nahm M.** 2011. Older adults have a low capacity to opsonize pneumococci due to low IgM antibody response to pneumococcal vaccinations. Infect Immun **79:**314–320. http://dx.doi.org/10.1128/IAI.00768-10.
42. **Vidarsson G, Sigurdardottir ST, Gudnason T, Kjartansson S, Kristinsson KG, Ingolfsdottir G, Jonsson S, Valdimarsson H, Schiffman G, Schneerson R, Jonsdottir I.** 1998. Isotypes and opsonophagocytosis of pneumococcus type 6B antibodies elicited in infants and adults by an experimental pneumococcus type 6B-tetanus toxoid vaccine. Infect Immun **66:**2866–2870.