**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-naïve Burkinabé and UK subjects were matched for age (1 to 39 years) and anti-Sp1 IgG level, analyzed for OPA to 3 type-specific 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.

*S. 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 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.
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-naïve 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 (Prevenar 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 (NTC) 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
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 \( \geq 8 \)) 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’Éthique 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 (\( \geq 0.35 \) g/ml, \( 0.35 \) to 1 g/ml, and \( >1 \) g/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 \( \geq 0.35 \) \( \mu \)g/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 \( \geq 8 \) 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 \( \geq 8 \) 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...
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 \( \leq 4 \) 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 \( \geq 8 \) 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 \( \beta \), 0.51 and 0.2; \( P \) values, 0.019 and 0.04 for IgM and IgG, respectively).
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 $\beta = 0.42$, $P = 0.024$).

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 ($\geq 8$); the dotted lines indicate the two IgG putative protection levels ($0.35$ and $1 \mu g/ml$). The blue circle indicates discrepant results with nonfunctional IgG.

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], 0.54 [0.46 to 0.63] among the youngest children (<5 years), 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 = 0.29). 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 Burkina Faso 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 Burkina Faso 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 Burkina Faso 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 Burkina Faso 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

| Serotype Measurement | Low IgG (<1 μg/ml) | High IgG (≥1 μg/ml) | P     |
|----------------------|-------------------|---------------------|-------|
| 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  | NA²   |
|                      | 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    |       |

Individuals were matched for serotype-specific OPA values between the two groups.

GM, geometric mean; CI, confidence interval.

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
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

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.
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 SPI 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 SPI 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 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-naïve 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.

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