Serological surveillance has been used in the United Kingdom to inform vaccine policy for several infections, including those with group C meningococci. Meningococcal conjugate vaccines, containing capsular groups A, W135, and Y in addition to C, are now available, but their use in the United Kingdom is restricted to at-risk groups and travelers to areas of endemicity. The aim of this study was to establish a baseline for natural immunity for groups W135 and Y. Serum samples collected in 2009 from individuals of all ages were obtained from the Health Protection Agency Seroepidemiology Unit, which collects residual sera from participating laboratories across the country. Serum bactericidal antibody (SBA) activity against two reference strains, representing groups Y (strain M03 241125) and W135 (strain M01 240070), was determined with 1,191 sera using a standardized complement-mediated SBA assay, with complement derived from baby rabbits (rSBA). The age-specific geometric mean titers (GMTs) and percentages of individuals with rSBA titers of ≥8 were calculated, together with 95% confidence intervals (CI).

Overall, 18.4% and 19.6% had rSBA titers of ≥8 for groups W135 and Y, respectively. Antibody prevalence varied by age. In general, rSBA titers were low for younger children, with serum samples from 7% and 13% of children under 5 years achieving titers of ≥8 against groups W135 and Y, respectively. GMTs peaked for 20- to 24-year-olds for group W135 (GMT, 7.1; 95% CI, 4.7, 10.9) and for 30- to 44-year-olds for group Y (GMT, 8.6; 95% CI, 5.9, 12.7). Unlike seroprevalence against group B meningococci, there was not an obvious peak in SBA titers in samples from teenagers. Natural immunity against group W135 and Y meningococci in England appears to be low.

Meningococcal group C conjugate (MenC) vaccines have been in use in the United Kingdom for more than 10 years and have successfully controlled group C disease (11). Serological studies were essential for their licensure and have been useful in interpreting vaccine impact and antibody persistence (8, 10, 30, 32). New quadrivalent conjugate vaccines are now available, offering protection against groups A, C, W135, and Y, but they have not been recommended for routine use in the United Kingdom. The incidence of laboratory-confirmed meningococcal disease is currently around 2 per 100,000, and these surveillance data indicate that group B remains by far the most common cause, now accounting for more than 85% of cases. There are a relatively small number of cases of W135 and Y disease, with 19 (2% of all cases) and 63 (7% of all cases) laboratory-confirmed cases in 2009-2010 (http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1234859711901), although the number of group Y cases has increased from around 30 per year in the middle of the decade. The aim of this study was to establish a baseline seroprevalence profile for groups W135 and Y in order to improve our understanding of the extent of natural exposure and immunity to these organisms. We also examined the association between seroprevalence and disease incidence by age to investigate whether the inverse relationship between the two, as described in the classic study of Goldschneider et al. (16), could be observed.

**MATERIALS AND METHODS**

Serum samples collected in 2009 from individuals of all ages were obtained from the Health Protection Agency Seroepidemiology Unit, which collects residual sera from routine diagnostic testing from participating laboratories across the country (26). There is no record of the indication for blood testing, but immunocompromised individuals are excluded (http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1226652136464). On the basis of previous experience, we aimed to test around 100 sera from each of the following age groups: <1 year, 1 to 2 years, 3 to 5 years, 6 to 9 years, 10 to 12 years, 13 to 15 years, 16 to 19 years, 20 to 24 years, 25 to 29 years, 30 to 44 years, 45 to 64 years, and 65+ years. We selected finer age bands in the ages likely to be considered for vaccination, i.e., young children and teenagers. In total, 1,191 serum samples were tested (Table 1 shows age-stratified numbers), but there were insufficient sera from infants to achieve the target sample size (n = 55).

Serum bactericidal antibody (SBA) activity against standard reference strains for groups Y (strain S1975/M03 241125, Y:2a:P1.5,2, O-acetyl negative) and W135 (strain M01 240070, W135:NT:P1.18-13, O-acetyl positive) was determined using a standardized complement-mediated SBA assay, with complement derived from baby rabbits (rSBA), as previously described (22). rSBA titers of <4 were assigned a value of 2 for computational purposes. The age-specific geometric mean titers (GMTs) and percentages of individuals with rSBA titers of ≥8 were calculated, together with 95% confidence intervals (CI). Although the evidence that an rSBA titer of ≥8 is protective has not been robustly established for groups Y and W135, as it has for group C meningococci (9), this has been used as a cutoff in studies evaluating the immunogenicity of quadrivalent conjugate vaccines (for example, see references 2 and 27). Disease incidence data were obtained from the Health Protection Agency. For each capsular group, the average annual incidence over four epidemiological years combined (2006-2007 to 2009-2010) is presented. Ethical approval for the seroepidemiology collection (for use in informing the national immunization program) was obtained from the South-West Thames Ethics Committee (07/Q1304/105). We also submitted the study protocol to Animal Ethics Committees for approval to use baby rabbits (School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom; Vaccine Evaluation Unit, Health Protection Agency Public Health Laboratory, Manchester, Manchester Royal Infirmary, Manchester, United Kingdom; and University of Manchester, Inflammation Sciences Research Group, School of Translational Medicine, Manchester, United Kingdom).
zation program for England and Wales) was granted by the Joint UCL/ UCLH Committees on the Ethics of Human Research (Research Ethics Committee [REC] reference number 05/Q0505/45).

RESULTS

The prevalence of rSBA varied by age for both groups Y and W135. The age-specific profile of GMTs is shown in Table 1, and the prevalence of rSBA titers of ≥8 is illustrated in Fig. 1. For group Y, GMTs peaked in the 30- to 44-year age group, and although 95% CIs overlapped for many age groups, rSBA GMTs were significantly higher (at the 5% level) for 30- to 44-year-olds than for those over 45 years of age (Table 1). The percentage of sera with titers of ≥8 was 19.6% over all ages and peaked in 30- to 44-year-olds at 35.2% for group Y (Fig. 1), significantly higher than that for those with ages of less than 15 years or more than 45 years. For group W135, GMTs peaked in the 20- to 24-year age group and were significantly higher in this age group than for those under 12 years of age (Table 1). The prevalence of rSBA titers of ≥8 for W135 was 18.4% over all ages and peaked for 25- to 29-year-olds at 28.0%, although this was only significantly higher than that for the 1- to 2-year age group (Fig. 1).

The relationship between disease incidence and seroprevalence of rSBA titers of ≥8 is shown in Fig. 2, with disease incidence compared to rSBA GMTs in Fig. 3. A clear inverse relationship between disease incidence and seroprevalence was not observed for either group.

DISCUSSION

This seroprevalence study has demonstrated that natural immunity against two reference strains representing capsular group W135 and Y meningococci in England varies by age, but in general the proportion of individuals with measurable immunity is low, with less than 20% of our sample achieving rSBA titers of ≥8 overall for both groups. Despite this, the disease incidence for W135 and Y remains low, indicating either modest circulation and/or low virulence of prevalent strains.

The classic Goldschneider curves showing in a clear inverse relationship between disease incidence and SBA activity (16) have not been reproduced for group Y or W135 here. This was the case when using both GMTs and a cutoff rSBA of ≥8 (Fig. 2) and was not improved when different cutoffs were used (data not shown), largely because a large proportion of sera were effectively "negative" (i.e., below the lower limits of detection of the assay). The SBA seroprevalence profiles for the two reference strains representing groups W135 and Y are more similar to the profiles of natural immunity observed for group C (before the introduction of MenC vaccination) (31) than for group B (33). In particular, for group B (where SBA activity was measured against B:4:P1.7-2,4, using human complement), we could clearly observe a peak in titers in serum from 15- to 24-year-olds (33) that corresponds

### TABLE 1 Geometric mean rSBA titers by age range of subjects with 95% confidence intervals for groups Y and W135

| Age group, yr(s) | Meningococcal group |  |  |
|-----------------|---------------------|--------------------------|
| Y               | W135                |  |  |
| No. of serum samples | GMT (95% CI) | No. of serum samples | GMT (95% CI) |
| <1              | 2.21 (2.04, 2.41)   | 3.00 (2.11, 4.27)       |
| 1–2             | 2.83 (2.24, 3.57)   | 2.54 (2.06, 3.13)       |
| 3–5             | 4.79 (3.29, 6.97)   | 3.36 (2.52, 4.47)       |
| 6–9             | 5.48 (3.75, 8.03)   | 3.53 (2.68, 4.65)       |
| 10–12           | 4.06 (2.87, 5.73)   | 3.43 (2.57, 4.59)       |
| 13–15           | 4.51 (3.06, 6.64)   | 3.78 (2.70, 5.29)       |
| 16–19           | 6.56 (4.13, 10.43)  | 3.89 (2.85, 5.30)       |
| 20–24           | 5.96 (3.89, 9.12)   | 7.13 (4.66, 10.90)      |
| 25–29           | 6.58 (4.31, 10.03)  | 6.23 (4.28, 9.08)       |
| 30–44           | 8.61 (5.86, 12.65)  | 5.70 (4.04, 8.06)       |
| 45–64           | 3.97 (2.86, 5.53)   | 5.22 (3.74, 7.30)       |
| 65+             | 3.74 (2.77, 5.06)   | 4.81 (3.47, 6.66)       |

FIG 1 Percentage of individuals with rSBA titers of ≥8 by age and capsular group (error bars represent 95% confidence intervals).

FIG 2 Percentage of individuals with rSBA titers of ≥8 against group Y (a) or W135 (b) compared to disease incidence per 100,000 by age.
Natural Immunity to Group Y and W135 Meningococci

with higher carriage rates in this age group (13). Although titer increase with age for W135 and Y, the peak is at older ages (>25 years) and is not striking, and overall seroprevalence is modest, suggesting that exposure to these capsular antigens through carriage is not common. This was also the case with group C prior to vaccination, but the ability of the ST-11 clonal complex to cause disease (as shown by the case/careter ratio) was much higher (12, 34).

This study used residual sera collected after routine diagnostic testing in participating laboratories. This convenience sampling method allows the collection and testing of large numbers of samples across the age range and has been used for a wide range of infections to inform public health policy (for example, see reference 23). Since we do not know the clinical indication for blood testing, we cannot be sure that the sera are representative of the general, healthy population; however, a study of immune responses to vaccine-preventable diseases that compared this method of sampling with random sampling showed very little difference (19). A further limitation of our methods is that we tested rSBA activity only against two reference strains, which may or may not be representative of the circulating W135 and Y strains in the United Kingdom. Our study measured serum bactericidal antibody activity against whole bacteria, and although the capsule is a major antigen, we cannot be sure what proportion of killing is antipolysaccharide mediated. Subcapsular antigens may also be important, and further research is required to investigate this.

Disease due to capsular group W135 has been historically low in the United Kingdom, with only a modest increase in disease in 2000 and 2001 due to an outbreak of W135 disease at the Hajj, Saudi Arabia. Of the 19,749 pilgrims from the United Kingdom who participated in Hajj 2000, 8 cases occurred, giving incidence rates of 41 per 100,000 (7). Pilgrims were affected first, followed by cases in household contacts, and then out-of-household contact cases and cases with no identifiable contact by week 19.

Three population-based W135 seroprevalence studies have been performed with African populations, two in the childhood and young adult populations in Burkina Faso in 2002 and 2003 and the third in Niger in 2003 among schoolchildren (3, 25, 28). These were largely prompted by the outbreak in 2002, where more than 10,000 cases caused by capsular group W135 isolates were reported from Burkina Faso (4, 14). The nasopharyngeal carriage rate of W135 organisms among healthy 5- to 25-year-olds residing in a district in Burkina Faso with an epidemic in 2002 was 25%, compared with 3.4% in a nonepidemic district (28). Seroprevalence of W135 also was higher in the epidemic district, with 60% having rSBA titers of ≥8, as opposed to 34% in the nonepidemic district, which likely reflected recent extensive exposure of the population (28). The percentage putatively protected in the study of Raghunathan et al. was higher than that found by Mueller et al. (25) in a different district of Burkina Faso a year later in 2003, which had experienced hyperendemic but not epidemic incidence of W135 disease. Here, 22% of 4- to 29-year-olds were found to have rSBA titers of ≥8. These data may indicate that substantial levels of protection against W135 are achieved only during epidemics and not in a situation where disease is endemic or hyperendemic.

The seroprevalence of W135 reported as baseline measurement from various clinical trials was summarized by Mueller et al. (24) and was found to vary more by country and study than by age group. This reflects differences in the assays used by different laboratories, in particular the target strains (5) and local conditions that lead to higher seroprevalence, specifically the degree of carriage and transmission and also the prevalence of locally occurring cross-reactive antigens. The above also holds true for capsular group Y, and a further potential factor is that the polysaccharide capsules for both groups Y and W135 can be in either an O-acetylated or de-O-acetylated form (20). For group W135, O-acetylation status was shown to impact serological measurements of antibodies directed against W135 in sera from immunized adults (15).

Capsular group Y invasive meningococcal disease in the United Kingdom is rare and until recently accounted for fewer than 2% of all meningococcal infections (17). In 2009, however, twice as many cases of invasive group Y disease were reported than in previous years (http://www.hpa.org.uk/web/HPAweb &HPAwebStandard/HPAweb_C/1234859711901). Of interest, a recent study has demonstrated that carriage due to group Y has increased significantly in university students in Nottingham, United Kingdom (1, 6), with up to 25% of healthy Group meningococci in winter 2009. These rates are substantially higher than the 5% carriage rate of group Y organisms for a multicenter carriage study performed in 1999, 2000, and 2001 with students of ages 15 to 19 years in the United Kingdom (21). The threat of expansion of group Y was highlighted by the increase in the proportion of disease caused by group Y strains in the United States in the early 1990s, where it rose from 2% in 1989 to 1991 (18) to 10.6% in 1992 and then to 32.6% in 1996 (29).

In conclusion, natural immunity to groups Y and W135 ap-
pears to be low, with a pattern of age-related seroprotection different from that with capsular group B. Careful monitoring of meningococcal disease should continue.

ACKNOWLEDGMENTS
This study was funded by a grant from the Meningitis Research Foundation (project 1001.0).
We thank Helen Chadha, Kelly Townsend, Dani Thompson, Lesley Mabey, Stephen Slater, Stephen Clark, and Kate Nolan from the Vaccine Evaluation Unit for performing the rSBA assays and Elaine Stanford for selecting the samples from the HPA Seroepidemiology Unit. Disease incidence data were kindly provided by Mary Ramsay and Helen Campbell, HPA Immunisation Department.
R.B. performs contract research on behalf of the Health Protection Agency for Baxter Bioscience, GSK, Merck, Novartis, Pfizer, Sanofi Pasteur, and Sanofi Pasteur MSD.

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