Mucosal Immunity in Healthy Adults after Parenteral Vaccination with Outer-Membrane Vesicles from *Neisseria meningitidis* Serogroup B

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Background. Nasopharyngeal carriage of meningococcus or related species leads to protective immunity in adolescence or early adulthood. This natural immunity is associated with mucosal and systemic T cell memory. Whether parenteral *Neisseria meningitidis* serogroup B (MenB) vaccination influences natural mucosal immunity is unknown.

Objectives. To determine whether parenteral MenB vaccination affects mucosal immunity in young adults and whether this immunity differs from that induced in the blood.

Methods. Otherwise healthy volunteers were immunized with MenB outer membrane vesicle vaccine before and after routine tonsillectomy. Mucosal and systemic immunity were assessed in 9 vaccinees and 12 unvaccinated control subjects by measuring mononuclear cell proliferation, cytokine production, Th1/Th2 surface marker expression, and antibody to MenB antigens.

Results. Parenteral vaccination induced a marked increase in systemic T cell immunity against MenB and a Th1 bias. In contrast, although mucosal T cell proliferation in response to MenB neither increased nor decreased following vaccination, mononuclear cell interferon γ, interleukin (IL)–5, and IL-10 production increased, and the Th1/Th2 profile lost its Th1 bias.

Conclusions. Parenteral MenB vaccination selectively reprograms preexisting naturally acquired mucosal immunity. As new-generation protein-based MenB vaccine candidates undergo evaluation, the impact of these vaccines on mucosal immunity in both adults and children will need to be addressed.

Neisseria meningitidis is a major cause of meningitis and septicemia in children and adolescents. The disease burden associated with serogroup B (MenB) infection in Europe, the United States, and South America is considerable [1, 2]. Serogroup C vaccine has been highly successful in several national vaccination programs, but of-

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fers no cross-protection. A specific glycoconjugate vaccine to prevent MenB infection is precluded by the high degree of homology between the capsular polysaccharide α-2,8-polysialic acid and brain carbohydrates [2, 3]. We argue that in order to inform the design of potential MenB vaccines and to improve the effectiveness of those under trial, there is a need to better understand the nature of mucosal immunity to meningococcus [4].

The immunological basis of the relationship between meningococcal commensalism and disease is ill defined. Infection with MenB follows nasopharyngeal carriage, but in most circumstances colonization is not associated with disease [5, 6]. Carriage rates vary from 5% to 40% in healthy people, whereas outside of periods when the disease is epidemic, 1–3 cases of infection are reported per 100,000 individuals. Most prevalent in people aged 19–24 years, periodic carriage of *N. meningitidis* or related species is thought to lead to the development of natural immunity [7, 8]. This view is supported by the correlation between increasing age, a decline in disease incidence, and the appearance of anti–*N. meningitidis* complement fixing antibody (serum bactericidal antibody [SBA]) [9]. However, a recent large serological survey suggests that other immune mechanisms may be important [8].

Our previous studies have demonstrated that in addition to the induction of SBA, natural immunity to MenB involves the generation of both mucosal and systemic memory T cell responses [10, 11]. We have shown that the mucosal T cell response is highly regulated by $CD4^+CD25^+$ cells and is Th1 polarized, whereas the systemic response is more evenly balanced between Th1 and Th2 memory [11]. Given the protective effectiveness of this immunity in adults, we suggest that vaccination strategies should attempt to mimic natural immunity in both the mucosa and the circulation [4].

The majority of MenB vaccines tested to date have been based on subcapsular protein antigen containing outer membrane vesicles (OMV), given by intramuscular injection [2]. In OMV, the immunodominant antigen is the porin PorA, which is highly variable between MenB strains [12–15]. Thus, eliciting an immune response against one variety of PorA frequently does not confer protection against strains with heterologous antigens. To overcome this obstacle, numerous different OMV-based vaccines have been extensively tested in trials [16–19]. A strain-specific OMV vaccine is currently being administered to New Zealanders to arrest a MenB epidemic [20], and novel antigens, including those discovered as a result of the sequencing of the meningococcal genome, are being tested in phase I and phase II trials [2, 21, 22]. Evidence indicates that although current systemic vaccination approaches engender 50%–90% protective efficacy in children and adults, this immunity is not broadly cross-protective and is often short-lived in children [2]. Although MenB vaccination strategies aim to confer herd immunity through a reduction in nasopharyngeal carriage, little is actually known about the mucosal immune effects of these vaccines.

Vaccines that alter natural immunity could boost preexisting mucosal immunity, be ineffective, or leave the host more susceptible to infection. In the present study, we investigated the systemic and mucosal effects of vaccination with OMV-based vaccine on the natural immune response to MenB in adults. By vaccinating individuals prior to tonsillectomy, we determined the influence of vaccination on mucosal cellular immunity and determined whether this response is compartmentalized from the systemic circulation.

SUBJECTS, MATERIALS, AND METHODS

Subjects. Nine vaccinees (median age, 21.0 years [range, 18–34 years]; 7 females; 2 smokers) and 12 unvaccinated control subjects (median age, 20.5 years [range, 16–34 years]; 10 females; 3 smokers) were recruited from a group of otherwise healthy individuals with a history of recurrent tonsillitis who were undergoing tonsillectomy . Two additional vaccinees withdrew because their surgery was rescheduled. None of the participants had a history of meningococcal disease, MenB or influenza vaccination, or evidence of current tonsillar infection. Written consent was obtained from all subjects. The collection of samples and the research described complies with relevant guidelines and institutional practices (United Bristol Healthcare Trust Local Research Ethics Committee E5711; UK National Research Register N0264132546).

MenB immunization. Subjects were given 0.5 mL of MenB vaccine, injected into the deltoid muscle of the nondominant arm (MeNZB vaccine; provided by Novartis Vaccines, Sienna, and manufactured by the Norway National Institute of Public Health), on day 0 and day 42 (56 and 14 days prior to tonsillectomy) (figure 1*A*). It was not feasible to deliver all 3 doses of the standard vaccination regime prior to tonsillectomy. A third dose of vaccine was therefore administered after sample collection [23]. Each dose contained OMV from a New Zealand epidemic strain from 1998 (NZ98/254, B:4:P1.7–2,4) and aluminum hydroxide [20]. The vaccine did not contain thiomersal, neomycin, bovine products, or egg products. The OMV used in this vaccine expressed a variety of PorA closely related to the PorA types prevalent in the UK population.

Sample collection. For study subjects, saliva, serum, and citrated blood samples were obtained prior to each vaccination and before tonsillectomy (days 0, 42, 56, and 84) (figure 1*A*); for unvaccinated control subjects, all samples were obtained together at the time of tonsillectomy. The palatine tonsils were collected in decontamination media [10]. Saliva samples were obtained by use of a sterile sponge swab (Malvern Medical Developments) [24]. Both serum and saliva samples were stored at $-80°C$ until use.

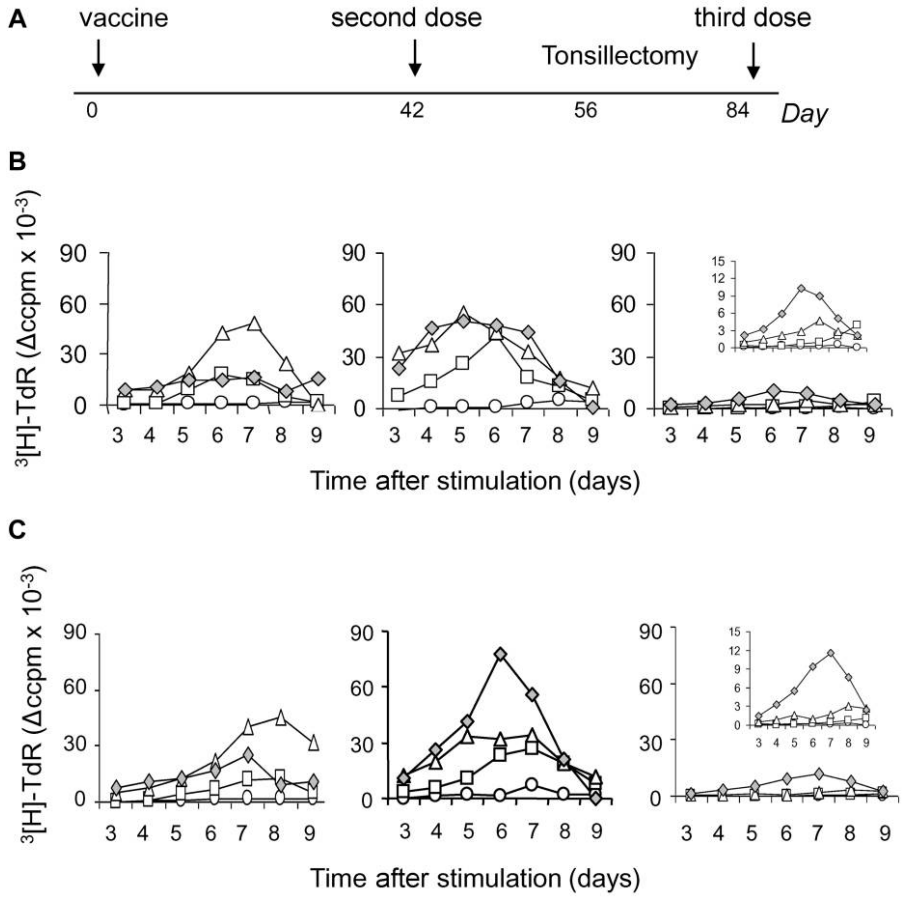

Figure 1. Timing of vaccination and proliferative kinetics of peripheral blood mononuclear cell (PBMC) responses toward vaccine outer membrane vesicles (OMV) antigens and PorA$^-$ OMV antigens in subjects vaccinated with *Neisseria meningitidis* serogroup B (MenB). *A,* Vaccination schedule. MenB vaccine was administered intramuscularly on days 0, 42, and 84 *(arrows).* Samples were taken just prior to vaccination and on day 56, just prior to tonsillectomy. Representative PBMC proliferative responses from 3 vaccinees after stimulation in vitro with vaccine OMV (B:4:P1.7-2,4) *(B)* and PorA$^-$ OMV (B:15:P1.$^{-,-}$) *(C).* The proliferative responses in media alone were subtracted to give corrected counts per minute (Δccpm) for samples obtained before vaccination *(circles)* and on days 42 *(squares),* 56 *(triangles),* and 84 *(diamonds).* Inserts provided to show trends where the height of response was lower. ^{3}[H]-Tdr, tritiated thymidine.

Meningococcal and control antigens. OMV were derived from the vaccine strain (NZ98/254, B:4: P1.7–2,4) and a spontaneous PorA- mutant (B:15:P1.−/−) of the Norwegian clinical invasive strain H44/76-SL (B:15:P1.7,16) by deoxycholate detergent extraction [10], as in previous studies of MenB immunity [10, 16, 18–20, 25]. Strains NZ98/254 and H44/76-SL are not isogenic and contain different PorB serotypes. Detergent extraction reduces the lipopolysaccharide (LPS) content from 25%–50% in native OMV to 5%–8% in detergent-extracted OMV. OMV were employed at concentrations of 0.1, 0.5, and 1 μg protein/mL. As a positive control for the proliferation experiments, we employed 0.2–1.8 μg/mL dialyzed inactive trivalent split virion influenza vaccine (Fluarix 2002/2003; GlaxoSmithKline) which contains no adjuvant [26].

Mononuclear cell isolation and culture. Single-cell suspensions of tonsillar mononuclear cells (TMCs) and peripheral blood mononuclear cells (PBMCs) were isolated [10]. Where required, CD45RA$^+$ cells were depleted by magnetic bead–associated cell sorting (MACS) by using CD45RA microbeads (Miltenyi Biotec) and LS$^+$ columns (Miltenyi Biotec) [10]. The efficiency of cellular depletion (>95%) was determined by flow cytometry. Cells were incubated at 37°C in 5% CO_2 with and without antigens for up to 9 days at 0.6×10^6, 1.0×10^6, and 1.5×10^6 cells/mL. PBMCs were diluted to 1×10^6cells/mL.

Proliferation assays. Tritiated-thymidine incorporation assays were used to monitor cellular proliferation over 9 days of culture with meningococcal and control antigens [10]. Background counts of cells in media alone were subtracted from antigen-stimulated cultures, and data were expressed as corrected counts per minute (Δccpm). Proliferation in response to MenB antigens in this assay has been previously been shown to be T cell mediated [10].

Phenotyping dividing memory T cells. To identify the dividing memory CD4$^+$ T cells in each compartment, CD45RO cells were labeled with carboxyfluorescein diacetate succinimidyl ester [10]. Labeled cells were then cultured with OMV (de-

rived from the vaccine strain or the PorA$^-$ strain) or media for 6 days. Samples of 10^6 cells were removed and stained with anti–CD4-APC (Dako) and either anti–interleukin (IL)-18R-phycoerythrin (R & D Systems) or anti–CRTH2-phycoerythrin (Miltenyi), and were analyzed by flow cytometry. Surface expression of IL-18R and chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2) was used to discriminate between Th1 and Th2 cells, respectively, within the responding populations [10].

Cytometric bead array (CBA). Culture samples (150 μL) were removed 3, 5, 7, and 9 days after stimulation and centrifuged (400 *g* for 5 min), and supernatants were stored at −80°C. Cytokine analysis was performed using a human Th1/Th2 CBA kit (BD Pharmingen) [26], in accordance with the manufacturer's instructions. Time-course experiments identified the peak day of cytokine production, as described elsewhere (Guthrie et al. [26]) (data not shown).

Flow cytometry. Analytical flow cytometry was performed by use of a FACSCalibur (BD Pharmacia). Cell Quest software was employed for data acquisition, and WinMDI (version 2.8; Joseph Trotter, Scripps Research Institute) was used for data analysis. Phenotypic acquisition was performed as described elsewhere [10], and analysis was restricted to assays in which >2% of CD45RO$^+$CD4$^+$ lymphocytes were responsive to MenB OMV above background. For CBA experiments, BD software, templates, and instructions were used to calculate concentrations of cytokines in each sample.

OMV ELISA for salivary and serum antibody. Specific salivary levels of IgA and IgG antibody against the vaccine OMV and PorA$^-$ OMV antigens were determined by ELISA [24]. Arbitrary values were calculated by using weighted probit regression analysis and an internal serum standard. Vaccine OMV–specific serum IgG endpoint titers were determined by ELISA, as described elsewhere [10, 18, 23].

Serum bactericidal antibody. SBA assays were performed against the vaccine strain, by use of methods described elsewhere with minor modifications [18, 23]. In brief, 25% human serum was used as an exogenous source of human complement. The starting dilution was 1:2, and titers were expressed as the reciprocal of the final dilution giving ≥50% bacterial lysis after 60 min of incubation. SBA titers of <2 were assigned a value of 1 for data analysis.

Statistical analysis. Triplicate data from proliferation assays yielded standard error values of <5%, which enabled the use of arithmetic means for subsequent statistical analysis. The Wilcoxon signed rank test was used to compare paired samples of PBMCs obtained before and vaccination; for the surface staining analysis, for which 1-way analysis of variance was used. The Mann-Whitney *U* test was used to compare unpaired data derived from cellular samples or serum samples. Statistical tests were performed using SPSS for Windows (version 11.0; SPSS). *P* values < .05 were considered to be statistically significant.

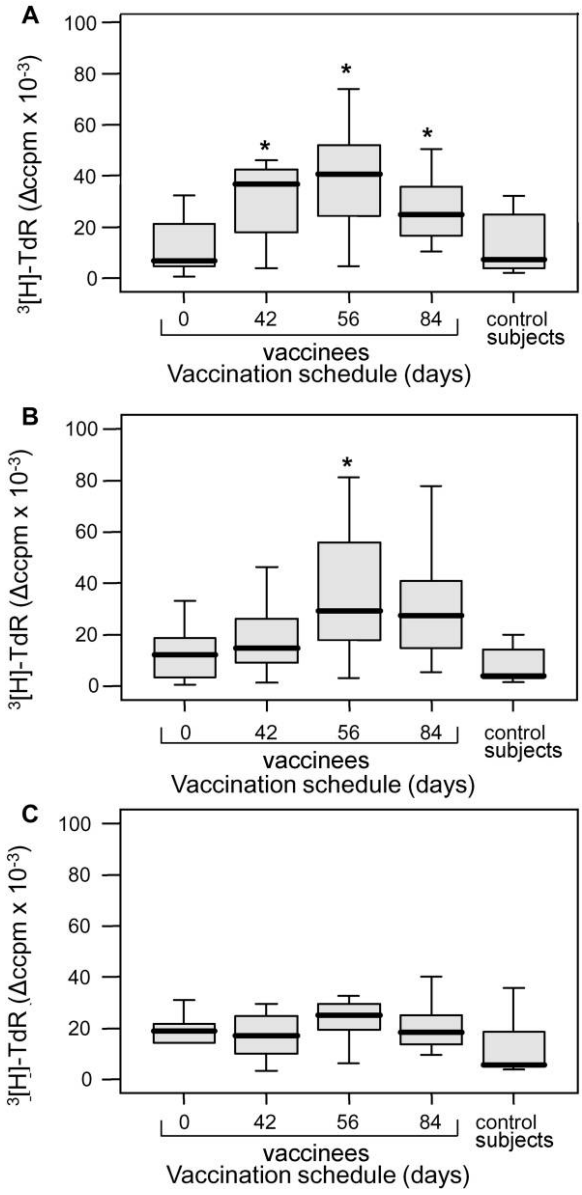

Figure 2. Peripheral blood mononuclear cell proliferation responses derived from control subjects (n=10) and vaccinated individuals (n=9) in response to vaccine outer membrane vesicles (OMV) antigen (B:4:P1.7-2,4) *(A),* PorA- OMV antigens (B:15:P1.$^{-,-}$) *(B),* and influenza antigens *(C).* Results are expressed as median peak proliferation *(black line)* of collated background subtracted and corrected responses (Δccpm), interquartile range *(box),* and full range *(error bars).* *P < .05. ^{3}[H]-Tdr, tritiated thymidine.

RESULTS

Peripheral proliferative responses to vaccination. Prior to vaccination, PBMC proliferative responses to MenB vaccine OMV in vitro were typically modest (figure 1*B*). As expected [25], strong proliferative responses to MenB vaccine antigens were observed after vaccination, even after a single dose of vaccine (day 42; 36,673 Δccpm [P < .01]). These responses generally exhibited the kinetics suggestive of T cell memory [10, 11],

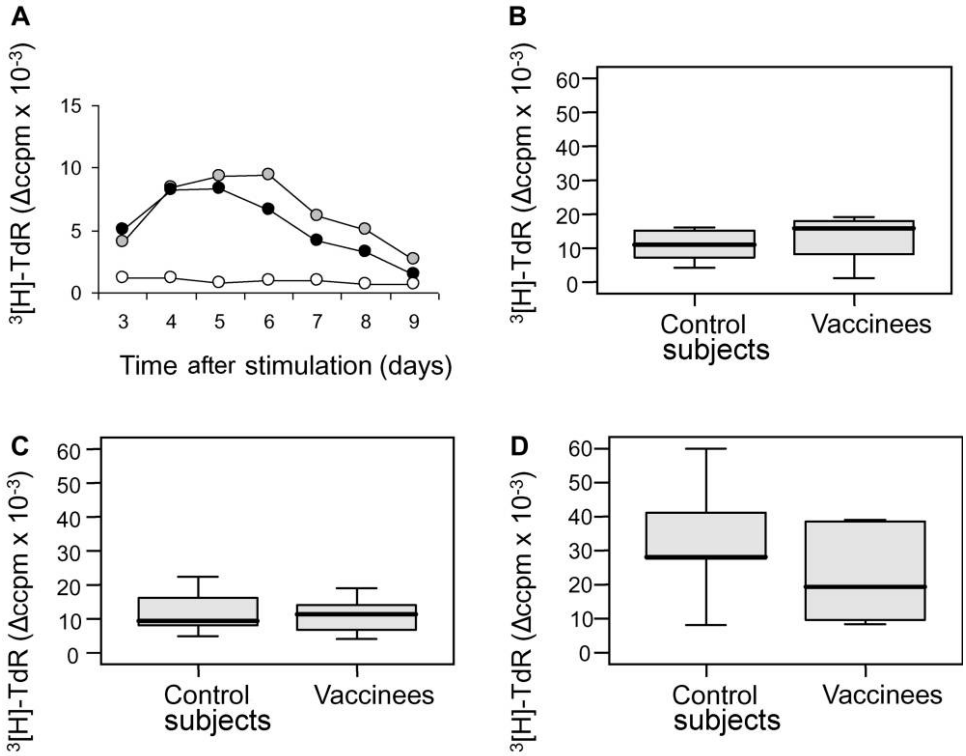

Figure 3. Mucosal mononuclear cell proliferation responses. *A,* Individual representative kinetics to vaccine outer membrane vesicle (OMV) antigen *(black filled circle),* PorA$^-$ OMV *(grey filled circle),* media control *(open circle). B–D,* collated peak proliferative responses of tonsillar mononuclear cells derived from vaccinated individuals (n=9) and unvaccinated controls (n=12) toward vaccine OMV antigens (B:4:P1.7-2,4) *(B),* PorA$^-$ OMV antigens (B:15:P1.$^{-,-}$) *(C),* and influenza antigens *(D).* Data are expressed as median background subtracted and corrected counts per minute (Δccpm) *(thick black line),* interquartile range *(box),* and full data range, *(error bars).* ^{3}[H]-Tdr, tritiated thymidine.

peaking around day 6 or 7 after vaccination, or earlier after 2 doses of vaccine (figure 1*B*). We have previously shown that a similar OMV vaccine induces T cell immunity to PorA and, to a lesser degree, to PorB [25]. In the present study, responses to MenB OMV from a different nonisogenic strain lacking PorA were markedly increased by vaccination (figure 1*C*) (day 56 [$P < .05$]). This indicates that vaccine-induced peripheral T cell responses were not restricted to PorA.

Proliferative responses to meningococcal antigens were highest 14 days after the second vaccine dose (on day 56) and had decreased by day 84 (figure 2*A* and 2*B*). Vaccination induced a more rapid and more sustained in vitro response to vaccine OMV, compared with PorA$^-$ OMV. As expected, vaccination did not influence the levels of in vitro reactivity to the control influenza virus antigens (figure 2*C*).

Mucosal proliferative responses to vaccination. As described elsewhere [10], naturally acquired immunity to MenB OMV was observed in cultures of TMCs from unvaccinated individuals. Responses usually peaked early (during days 5–7) (figure 3*A*), indicative of mucosal T cell memory [10, 11], and were equivalent in magnitude for each OMV, again highlighting the polyspecific nature of the response. Compared with responses in the unvaccinated group, responses in vaccinees showed that systemic vaccination did not alter the level of TMC proliferation in response to either MenB OMV (figure 3*B*) or the influenza control antigens (figure 3*C*).

Secretory cytokine profiles. To specifically investigate the nature of the T cell memory pool, cultures enriched for CD45RO$^+$ memory T cells (CD45RA$^+$ depleted) were stimulated for 7 days in vitro, and cytokine profiles were then analyzed by CBA. Considerable interindividual variation was observed. With respect to the mucosal compartment, comparison of cultures of cells from the unvaccinated control subjects and cultures of cells from vaccinated subjects showed a trend towards an increase in interferon (IFN)–γ, interleukin (IL)–5, and IL-10 production in response to either MenB vaccine OMV or PorA$^-$ OMV that was not significant ($P > .05$). There was also a trend towards a higher baseline level of mucosal IL-10 in unstimulated when cultures of cells from vaccinees were compared with cultures of cells from unvaccinated subjects (figure 4).

With respect to the systemic compartment, comparison of cultures of cells obtained before and after vaccination showed a marked increase in IFN-γ and IL-5 production, but no increase in IL-10 production, in response to either MenB vaccine OMV or PorA$^-$ OMV ($P < .05$) (figure 4). In both compartments,

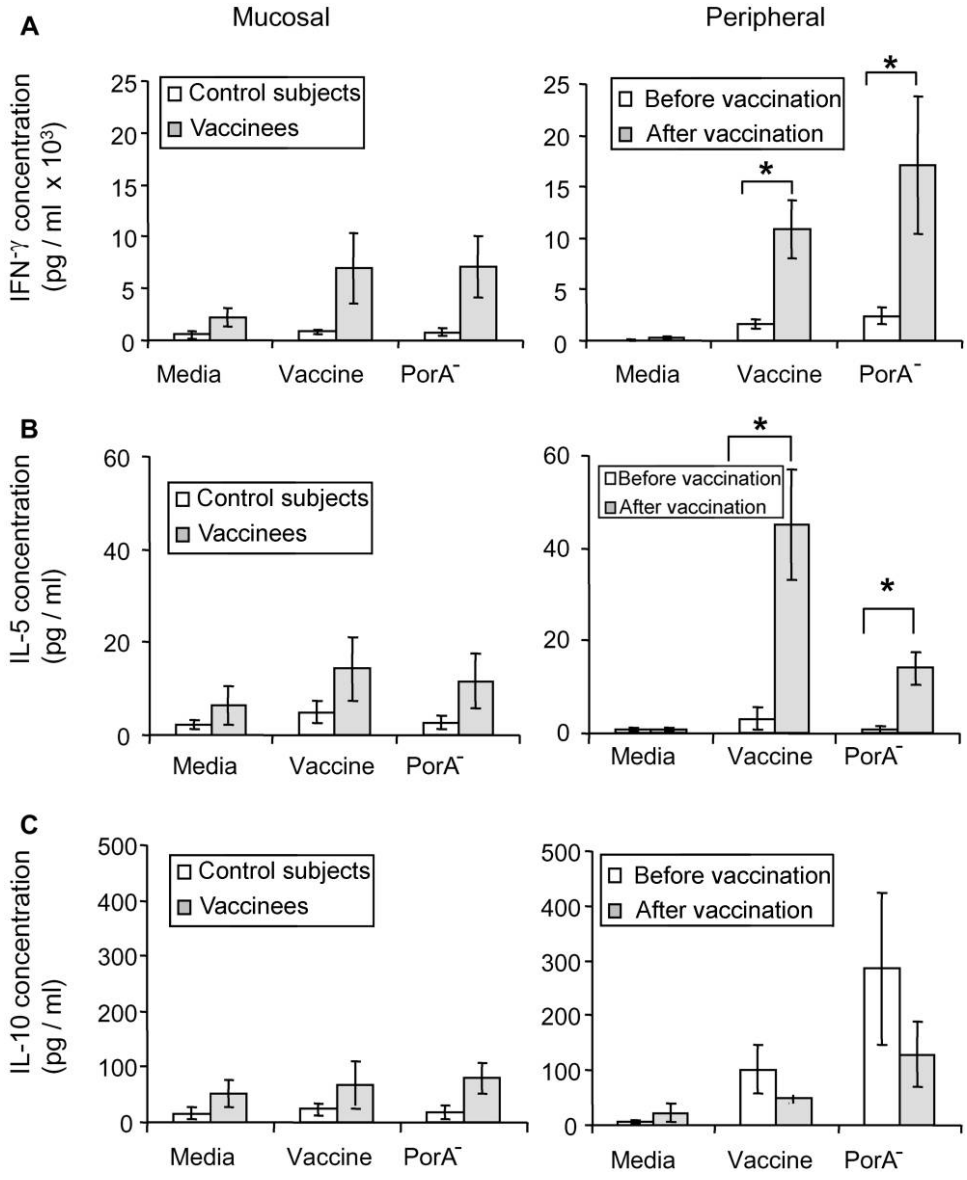

Figure 4. Cytokine profiles in cultures of tonsillar mononuclear cells (TMCs) and (PBMC) peripheral blood mononuclear cells. Cultures of $CD45RO^+$-enriched TMCs and PBMCs from vaccinees (TMCs from 7 vaccinees and PBMCs from 8 vaccinees) and unvaccinated control subjects (TMCs from 5 subjects and PBMCs from 8 subjects) were stimulated with vaccine outer membrane vesicle (OMV) and $PorA^-$ OMV). *A,* Interferon (IFN)–γ; *B,* Interleukin (IL)–5; and *C,* IL-10. Data are means and standard error. $^*P < .05$.

values for IL-2 and IL-4 were consistently below the threshold of detection (10 pg/mL) and tumor necrosis factor-α was not significantly altered by vaccination (data not shown).

Memory T helper cell phenotypes. Having identified a change in MenB OMV–induced cytokine expression in $CD45RO^+$ T cell–enriched cultures after vaccination, the phenotype of the responding memory $CD4^+$ cells was determined. As shown in a previous study [11], in the control group of unvaccinated subjects the naturally acquired mucosal response to MenB antigens was Th1 biased, as determined by cell-surface expression of IL-18R relative to expression of CRTH2 for paired data (figure 5*A*) ($P < .05$). In contrast, CD45RA-depleted cultures of TMCs from vaccinees demonstrated no significant bias of response (figure 5*B*). It was not feasible to obtain sufficiently large volumes of blood immediately prior to surgery to accurately phenotype systemic $CD45RO^+CD4^+$ T cells that responded specifically to vaccine OMV. However, after vaccination, peripheral blood $CD45RO^+CD4^+$ cells that responded to MenB exhibited a significant Th1 bias ($P < .05$ for comparison of IL-18R and CRTH2) (figure 5*C*), similar to the naturally acquired mucosal response observed in the absence of vaccination.

Serum and salivary antibody. Salivary levels of IgA and IgG against the vaccine strain were not altered by vaccination (figure 6*A* and 6*B*). There was also no change in the level of

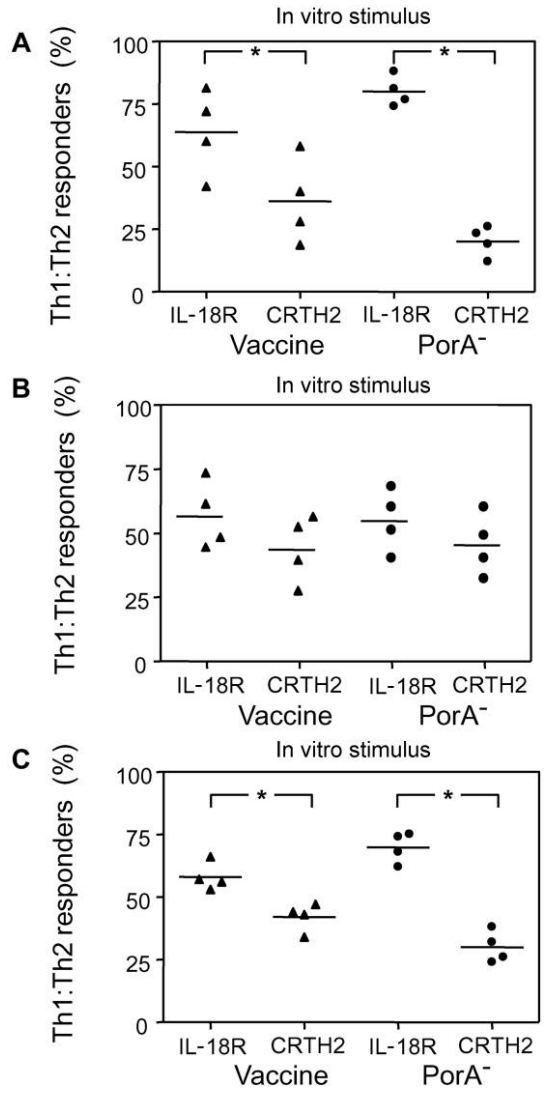

Figure 5. Ratio of T helper cell phenotypes responding to vaccine outer membrane vesicle antigens (OMV) and PorA⁻ OMV antigens in cultures of tonsillar mononuclear cells (TMCs) from unvaccinated control subjects (n=4) *(A),* TMCs from vaccinees (n=4) *(B),* and peripheral blood mononuclear cells from vaccinees (n=4) *(C).* Results represent the percentage expression of interleukin (IL)–18R and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) on the $CD4^+CD45RO^+$ memory lymphocytes that divided in culture (i.e., carboxyfluorescein diacetate succinimidyl ester–low cells) . $^*P < .05$.

mucosal antibody to the PorA⁻ strain (data not shown). In contrast, serum IgG titers against the vaccine strain increased significantly ($P < .05$) after a single dose of vaccine (day 42) (figure 6*C*). These levels remained elevated up to 42 days after the second vaccine dose (day 84), rising 10- to 12-fold on average ($P < .05$). Further analysis demonstrated that the vaccine-induced serum IgG was broadly cross-reactive against a panel of MenB strains representative of those in the United Kingdom and strongly cross-reactive (5-fold increase) with a UK strain of the same PorA subtype (B15: 1.7–2,4) (data not shown).

Three individuals were SBA positive (titer ⩾4) against the vaccine strain before immunization (subjects 2, 4, and 9). An SBA titer of ⩾4 has been tentatively proposed as protective [18, 23]. These subjects also had high IgG titers (>100 arbitrary

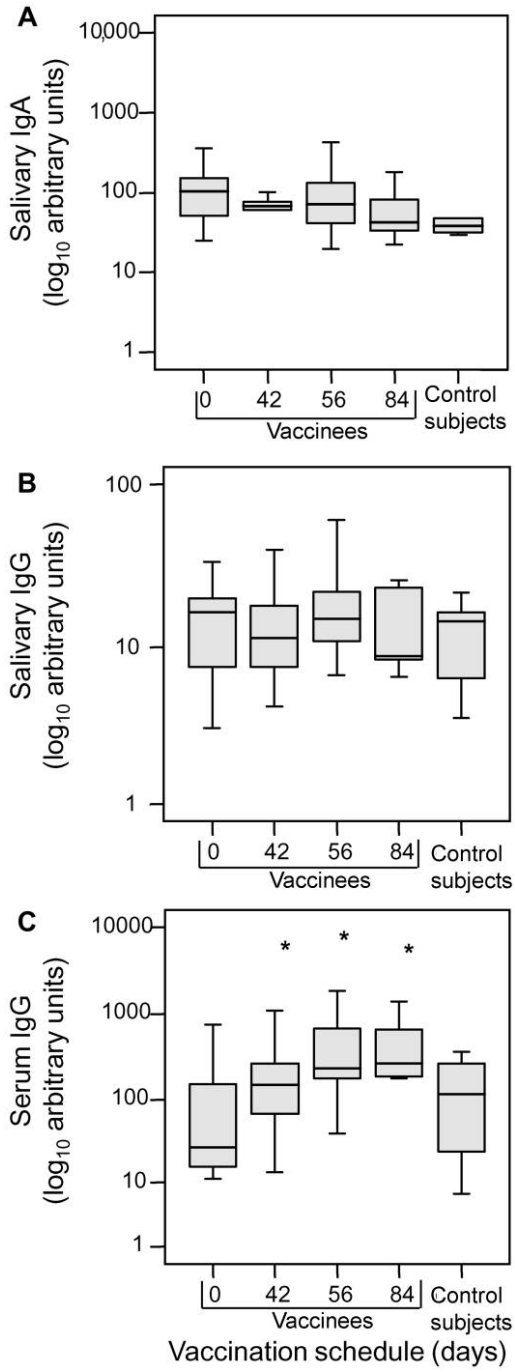

Figure 6. *Neisseria meningitidis* serogroup B–specific antibodies in serum ($n = 9$) and saliva ($n = 9$) before and after vaccination, as well as in unvaccinated control subjects (tonsillar mononuclear cells from 12 subjects and peripheral blood mononuclear cells from 10 subjects) analyzed by ELISA using vaccine outer membrane vesicles as the coating antigen. Graphs represent median titers *(black line),* interquartile range *(box),* and full data range *(error bars).* $^*P < .05$.

Table 1. Serum antibody responses in study subjects vaccinated against *Neisseria meningitidis* serogroup B.

	Before vaccination			After 2 doses of vaccine[a]			*N*-fold increase after 2 doses of vaccine[a]		
Subject number	SBA titer[b,c]	Serum IgG level, AU	Peak PBMC proliferation rate, Δccpm,	SBA titer[b,c]	Serum IgG, level, AU	Peak PBMC proliferation rate, Δccpm	SBA titer[b,c]	Serum IgG level, AU	Peak PBMC proliferation rate, Δccpm
1	1	11	13,958	16	184	31,528	16	16.4	2.3
2	64	154	4713	128	661	24,903	2	4.3	5.3
3	2	16	1465	32	178	16,501	16	11.1	11.3
4	4	198	21,278	8	197	13,921	2	1.0	0.65
5	1	27	32,344	2	332	24,903	2	12.3	0.77
6	1	13	23,194	1	25	35,661	1	2.0	1.54
7	1	20	6766	2	267	71,848	2	13.4	10.6
8	1	40	5035	16	1384	50,535	16	34.3	10.0
9	64	747	566	16	1092	10,260	1	1.5	18.1

NOTE. Responses to *N. meningitidis* serogroup B were determined by use of outer membrane vesicles derived from the vaccine strain. AU, arbitrary units; Δccpm, background subtracted and corrected counts per minute; PBMC, peripheral blood mononuclear cell; SBA, serum bactericidal antibody.

[a] Results for 42 days after the second dose of vaccine.

[b] Baseline SBA titers of ⩾4 and >4-fold increases after vaccination were considered protective.

[c] SBA titers of <2 were assigned a value of 1 for data analysis.

units) but there was no relationship with the magnitude of the PBMC proliferative response (table 1). All of the 9 vaccinees demonstrated preexisting SBA to at least 1 of 5 UK MenB strains tested (data not shown). Three subjects demonstrated strong elevation of SBA (16 fold) to the vaccine strain after 2 vaccine doses (subjects 1,3, and 8). These were accompanied by increases in the level of IgG (>10 fold) and the rate of PBMC proliferation (>2 fold). Four subjects became SBA positive against at least 1 UK strain during the course of the study (B:NT: 22,9: $n=3$; B:P1.7–2,4: $n=1$). Overall, there was no apparent relationship between mucosal T cell or salivary antibody responses and peripheral T cell, SBA or serum antibody responses.

DISCUSSION

Pathogens such as *N. meningitidis, Hemophilus influenzae* type b (Hib), and *Streptococcus pneumoniae* are highly adapted for colonization of their mucosal niche. The implementation of Hib, pneumococcal, and meningococcal serogroup C (MenC) conjugate vaccines has revealed that in addition to inducing individual protection, these highly effective vaccines generate herd immunity by limiting mucosal carriage and thus blocking person-to-person spread [27–29]. How MenB protein–based vaccines influence mucosal immunity is uncertain. In this study, we used systemic vaccination of adults who underwent routine tonsillectomy to probe the influence of vaccine on naturally acquired human immunity to MenB. We showed that parenteral vaccination induced a marked increase in systemic humoral and cellular immunity against MenB, as well as a Th1 bias. Although mucosal T cell proliferation in response to MenB antigens and the level of specific salivary IgA were unaffected by OMV vaccination, mononuclear cell production of IFN-γ, IL-5, and IL-10 increased, and the Th1 bias of MenB-specific memory T cells was lost. The changes in immunity after vaccination that were seen in both compartments followed in vitro stimulation with either $PorA^+$ or $PorA^-$ OMV. This suggests that vaccine-induced cellular immunity was induced not just by immunodominant PorA [12–14, 25, 30] but also by other surface protein antigens that may be shared between MenB strains [12, 25].

Compartmentalization of MenB-specific T cell immunity associated with natural exposure [10, 11] was maintained after vaccination. Changes in the mucosa may have resulted from either peripheral memory cell generation and subsequent mucosal homing or active migration of proinflammatory responders from the site of natural carriage to the periphery where the vaccine was introduced. Although both are possible, the absence of a reduction in mucosal T cell proliferation after vaccination and an increase in both proinflammatory and immunosuppressive cytokines [31] suggest active reprogramming of the mucosal compartment. How this immunological redirection was mediated by vaccination is uncertain. MenB vaccine OMV express an array of molecular patterns that signal via Toll-like receptors including LPS, which is a major component of the gram-negative outer membrane and has potent adjuvant activity [32–37]. LPS activates dendritic cells and enhances uptake of meningococcal antigens [38, 39], and it could account for the immunomodulatory effects of vaccination and the apparent Th1 bias in the blood after vaccination. However, our earlier studies suggest that the presence or absence of LPS in culture does not influence the phenotype of fully committed MenB-specific memory T cells responding to MenB antigens [10, 11]. The dif-

ferential effects of colonization and vaccination with respect to their mechanisms for immune programming remain to be determined, but our findings emphasize the importance of the site of induction to this process.

The type of immune profile in both compartments following MenB vaccination may have important consequences for immune protection. Although a Th1-skewed immune response in the blood will promote SBA, a more balanced response will enhance other components, such as T cell memory, T cell diversity, IgA production, and opsonophagocytosis [40, 41]. Recent studies of *Helicobacter pylori* and *S. pneumoniae* infection in animals have shown that mucosal clearance after vaccination with protein-based vaccines is independent of B cell function [42, 43]. Mucosal Th1-dominated immunity is mediated through activation of macrophages and NK cells, enhanced FcγR1 expression on phagocytes, neutrophil mobilization, and induction of complement-fixing and opsonizing antibodies [44, 45]. Thus a loss of Th1 bias in the mucosal compartment may compromise protection against carriage and invasion. Indeed, although we have previously demonstrated that mucosal IgA is reactive against a range of UK MenB outer membrane proteins in children and adults [24], in this and another vaccine study [23] no elevation of salivary IgA level was detected following MenB vaccination.

The appearance of IL-10 in mucosal cell cultures after vaccination may reflect a regulatory profile [31]. We have previously demonstrated that depletion of $CD25^+$ cells reveals suppression of the effector $CD4^+$ T cell response by regulatory T cells restricted to the mucosa [11]. Thus, systemic vaccination has the potential to unmask or enhance IL-10–mediated $CD4^+CD25^+$ regulatory activity in the human mucosa in the context of preexisting natural immunity. This regulatory activity may have a range of effects on both innate and adaptive immunity [46, 47]. Intranasal vaccination with MenB OMV vaccines induces local IgA but results in fewer vaccine responders in the blood than systemic vaccination [48–50]. The regulatory effects of vaccination and the influence of the route of administration are currently under investigation.

Our observations are limited by the use of material taken from subjects with tonsillar enlargement and recurrent tonsillar infection. However, we maintain that this approach provides a unique window into an important site of immune induction and surveillance for the meningococcus. None of the subjects had clinical tonsillitis at the time of the study, immunohistochemical studies did not reveal marked neutrophil infiltration or necrosis [11], and the tissue was immunologically responsive to experimental stimuli.

In conclusion, our data suggest that MenB OMV vaccine selectively reprograms preexisting naturally acquired immunity to colonizing bacteria at the mucosal surface. Whether this immunity will protect against carriage remains to be seen and will only become clear in either very large phase III studies or national vaccine implementation studies. In the meantime, new-generation OMV or protein-based vaccine candidates are undergoing further evaluation [2, 21, 22]. The impact of these vaccines on naturally induced mucosal immunity will need to be further investigated.

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