MAJOR ARTICLE

Differences in Homing Potentials of *Streptococcus pneumoniae*–Specific Plasmablasts in Pneumococcal Pneumonia and After Pneumococcal Polysaccharide and Pneumococcal Conjugate Vaccinations

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Background. Mucosal immune mechanisms in the upper and lower respiratory tracts may serve a critical role in preventing pneumonia due to *Streptococcus pneumoniae*. *Streptococcus pneumoniae*–specific plasmablasts presumably originating in the lower respiratory tract have recently been found in the circulation in patients with pneumonia. The localization of an immune response can be evaluated by exploring homing receptors on such plasmablasts, yet no data have thus far described homing receptors in pneumonia.

Methods. The expression of $\alpha_4\beta_7$, L-selectin, and cutaneous lymphocyte antigen (CLA) on *S. pneumoniae*–specific plasmablasts was examined in patients with pneumonia (n = 16) and healthy volunteers given pneumococcal polysaccharide vaccine (PPV; n = 14) or pneumococcal conjugate vaccine (PCV; n = 11).

Results. In patients with pneumonia, the proportion of *S. pneumoniae*–specific plasmablasts expressing L-selectin was high, the proportion expressing $\alpha_4\beta_7$ was moderate, and the proportion expressing CLA was low. The homing receptor $\alpha_4\beta_7$ was expressed more frequently in the pneumonia group than in the PPV ($P = .000$) and PCV ($P = .029$) groups, L-selectin was expressed more frequently in the PPV group than in the PCV group ($P = .014$); and CLA was expressed more frequently in the pneumonia group than in the PPV group ($P = .001$).

Conclusions. The homing receptor profile in patients with pneumonia was unique yet it was closer to that in PCV recipients than in PPV recipients. These data suggest greater mucosal localization for immune response in natural infection, which is clinically interesting, especially considering the shortcomings of vaccines in protecting against noninvasive pneumonia.

Keywords. pneumonia; *Streptococcus pneumoniae*; pneumococcus; pneumococcal polysaccharide vaccine; PPV; pneumococcal conjugate vaccine; PCV; mucosal immune response; lymphocyte homing; homing receptor.

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Pneumonia is a major cause of death worldwide, with *Streptococcus pneumoniae* one of the most frequent pathogens [1, 2]. Control of the disease through vaccination has become an objective for preventive health care. The disease begins by the establishment of *S. pneumoniae* colonization in the upper respiratory tract (URT), followed by descent of the bacteria into the lower respiratory tract (LRT) to bring about a symptomatic infection [1, 2]. Nasopharyngeal carriage is clearly a prerequisite for all *S. pneumoniae* diseases [2], yet the immunological factors protecting against transition from carriage to disease are not adequately understood.

Mucosal antibodies capable of preventing colonization may offer a key approach to protection [3–7]. Indeed, in animal models mucosal vaccines eliciting mucosal antibodies have proved to confer protection against local disease [5, 7, 8]. To develop better vaccination strategies, local immune mechanisms need to be explored in greater detail in humans.

Pneumococcal vaccines, either as 23-valent polysaccharide preparations (PPV) or 10- or 13-valent polysaccharide vaccines conjugated to a protein carrier (PCV10 and PCV13, respectively), are licensed in many countries. PPV covers a broader spectrum of serotypes and confers protection against invasive pneumococcal disease, while the efficacy against local, noninvasive pneumonia remains controversial [9–11]. PCVs cover fewer serotypes and are not only effective against invasive pneumococcal disease [12], but also seem to confer some protection against noninvasive pneumonia (efficacy, 20%–37%) [13–15]. The insufficient ability of vaccines to elicit mucosal immune response has been suggested to account for their shortcomings in providing protection against local disease [5].

During an immune response, effector B lymphocytes (plasmablasts) do not spread equally across the body but instead are guided to travel to sites of expected antigen encounter. This tissue-specific homing of lymphocytes from blood into tissues is based on interaction between lymphocyte surface chemokine receptors (CCRs) and homing receptors with chemokines and specific homing receptor-ligands, addressins, on the endothelial cells of the target tissues, respectively [16, 17]. Tissue-specific homing receptors have been identified: L-selectin (CD62L) guides the lymphocytes to the peripheral lymph nodes [18], $\alpha_4\beta_7$-integrin guides lymphocytes to the intestinal lamina propria [19], and cutaneous lymphocyte antigen (CLA) guides lymphocytes to skin tissue [20]. Analysis of the homing receptor and/or CCR profiles of the circulating plasmablasts provides an approach to evaluate the targeting of an immune response elicited at the site of antigen encounter [21–24]. The homing receptors guiding lymphocytes to the LRT have not been identified, but it has been suggested that instead of a single homing receptor, a variety of homing receptors are involved [16, 17, 25].

We have recently detected *S. pneumoniae*–specific plasmablasts in the circulation of humans with acute pneumococcal pneumonia [26]. These cells are presumed to originate in the LRT and to be responsible for distributing the immune response to various immunological compartments of the body [26]. Exploring these *S. pneumoniae*–specific plasmablasts allows study of the localization of the immune effector cells of the LRT. Comparing the homing receptor profiles of *S. pneumoniae*–specific plasmablasts between patients with pneumonia and volunteers who receive parenteral *S. pneumoniae* vaccines may throw new light on possible differences between natural infection and PPV or PCV in targeting the immune response.

METHODS

Patients, Healthy Volunteers, Vaccines, and Samples

The study protocol was approved by the Ethics Committee of the Department of Medicine at the Helsinki University Hospital. Written informed consent was obtained from all subjects. The study was registered at ClinicalTrials.gov with the identifier NCT01402245.

The 16 patients (8 women and 8 men aged 30–67 years) with acute pneumonia caused by *S. pneumoniae* composed the same group presented in our recent study [26] who had pathogen-specific antibody-secreting cells (ASCs) in peripheral blood specimens one week after the onset of symptoms. The diagnostic criteria are presented in that report.

A total of 14 healthy, previously unimmunized volunteers (9 women and 5 men aged 25–48 years) were enrolled in the PPV group, and 11 healthy, previously unimmunized volunteers (7 women and 4 men aged 24–40 years) were enrolled in the PCV group. The PPV group was vaccinated with Pneumovax (Sanofi Pasteur MSD, Brussels, Belgium), a 23-valent commercial PPV in which each dose contains 0.025 mg of capsular polysaccharide of serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. The PCV group was given Prevenar (Pfizer Inc. [previously Wyeth Lederle Vaccines]), a 7-valent PCV containing 0.002 mg of capsular polysaccharide of serotypes 4, 9V, 14, 18C, 19F, and 23F conjugated with CRM_{197} protein and 0.004 mg of serotype 6B conjugated with CRM_{197} protein. Each vaccinee received 0.5 mL of Pneumovax or Prevenar into the left deltoid muscle on day 0.

A blood sample was collected from patients with pneumonia 7–10 days after symptom onset and from vaccinees on days 0 and 7 after vaccination. This timing was based on our previous studies of mucosal [23, 27, 28] and parenteral [22] vaccination: following antigen encounter, ASCs appear in the circulation on days 2–3, peak on day 7, and are no longer found on day 14. Peripheral blood mononuclear cells (PBMCs) isolated using Ficoll-Paque and fractionated into receptor-positive and receptor-negative populations were analyzed with an enzyme-linked immunospot (ELISPOT) assay to determine the numbers of *S. pneumoniae*–specific ASCs and all immunoglobulin-secreting cells (ISCs). The ELISPOT assay identified separately the cells of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) isotypes.

Antigen

In the pneumonia group, *S. pneumoniae* isolates from cultures of blood specimens obtained from each patient were grown on chocolate agar plates, killed with formalin, and used as antigen in the ELISPOT assay, as described previously [29, 30]. In the ELISPOT assay for vaccinated volunteers, purified pneumococcal capsule polysaccharide 14 (ATCC; Bethesda, Maryland) was used as antigen. To observe possible differences between whole

bacteria and capsular polysaccharide as antigens, 1 representative patient with pneumonia was analyzed by the ELISPOT assay, using the *S. pneumoniae* strain recovered from the patient, and by another ELISPOT assay performed with a mixture of purified capsular polysaccharides (23F, 3, 4, 5, 6B, 7F, 8, 14, and 19F [ATCC]; 10 μg/mL of each type).

Separation of Receptor-Negative and Receptor-Positive Cell Populations

Separation of PBMCs into homing receptor–positive and homing receptor–negative populations has been described earlier [22, 29, 31]. Briefly, aliquots of cell suspensions were incubated on ice with antihuman $\alpha_4\beta_7$ (ACT-1), antihuman L-selectin (Leu 8), or antihuman CLA (HECA-452) and washed. Next, the cells were incubated on ice with Dynal M-450 magnetic beads coated with sheep antimouse IgG, followed by magnetic separation. The receptor-positive and receptor-negative cell populations were immediately studied by the ELISPOT assay.

ELISPOT Assay of Specific ASCs and All ISCs

The ELISPOT assay for pathogen-specific ASCs has been described previously [29]. In brief, 96-well microtiter plate wells were coated with a whole-cell preparation of the *S. pneumoniae* strain from each patient or, for vaccinees, with pneumococcal capsule polysaccharide 14. The cells were incubated in the wells for 2–3 hours, and antibodies secreted during this time were detected with alkaline phosphatase–conjugated antihuman IgA, IgG, and IgM. The substrate was added in melted agarose. Enumerated under a light microscope, each spot was interpreted as a print of 1 ASC.

The receptor-positive and receptor-negative cell populations were assayed for all ISCs, using the ELISPOT as previously described [32].

Statistical Analysis

The numbers of *S. pneumoniae*–specific ASCs for individual subjects were obtained by totaling their receptor-positive and receptor-negative ASCs in the 3 receptor assays and calculating the mean of these values. The total ASC numbers in each group were presented as median values with ranges. The data were log transformed and proved to be normally distributed; the differences between the study groups were assessed by analysis of variance.

The proportion of ASCs expressing a given receptor was given as the arithmetic mean ± standard deviation (SD). To obtain reliable statistics, we included in the homing receptor analyses only values based on ≥20 ASCs initially identified by the ELISPOT assay. The proportion (%) of receptor-positive cells among ASCs was calculated as follows: [(no. of ASCs in receptor-positive population)/(total no. of ASCs in receptor-positive and receptor-negative populations)] × 100. The proportion (%) among ISCs was calculated as follows: [(no. of ISCs in receptor-positive population)/(total no. of ISCs in receptor-positive and receptor-negative populations)] × 100.

The Shapiro–Wilk test showed that the homing receptor data were not normally distributed in all groups. The differences between groups were examined using the independent-samples Kruskal–Wallis test, followed by a post hoc test applying the Dunn-Bonferroni method, which takes into account multiple testing (SPSS, version 21.0). Differences were considered statistically significant when the P value was <.05.

RESULTS

Pneumococcal Isolates in Patients with Pneumonia

The patients' *S. pneumoniae* isolates were serotyped at the reference laboratory of the Institute of Health and Welfare. Each strain was determined to be serotype 4, 6B, 7F, 9V, 14, or 23F.

General Characteristics of ASC Responses

The magnitude of the *S. pneumoniae*–specific response (IgA+IgG+IgM-ASCs) was similar in the 3 study groups. In the pneumonia group, the median total number of pathogen-specific ASCs (IgA+IgG+IgM) was 152/10^6 PBMCs (range, 22–2039 ASCs/10^6 PBMCs; Figure 1*A*), and the median total number was 12 ASCs/10^6 PBMCs (range, 0–1235 ASCs/10^6 PBMCs) for IgA-ASCs, 119 ASCs/10^6 PBMCs (7–749 ASCs/10^6 PBMCs) for IgG-ASCs, and 5 ASCs/10^6 PBMCs (0–176 ASCs/10^6 PBMCs) for IgM-ASCs.

No *S. pneumoniae*–specific ASCs were found in the healthy volunteers before vaccination (Figure 1*B* and 1*C*). On day 7, *S. pneumoniae*–specific ASCs were detected in all subjects in both vaccination groups. The median count was 491 ASCs (IgA+IgG+IgM)/10^6 PBMCs (range, 92–8327 ASCs/10^6 PBMCs) in the PPV group (Figure 1*B*) and 265 ASCs/10^6 PBMCs (range, 43–812 ASCs/10^6 PBMCs) in the PCV group (Figure 1*C*).

As for the isotype distribution, data on the pneumonia group have been reported previously [26]. In the PPV group, the median values for *S. pneumoniae*–specific IgA-ASCs, IgG-ASCs, and IgM-ASCs were 199 ASCs/10^6 PBMCs (range, 20–2955 ASCs/10^6 PBMCs), 182 ASCs/10^6 PBMCs (range, 9–3575 ASCs/10^6 PBMCs), and 15 ASCs/10^6 PBMCs (range, 0–1798 ASCs/10^6 PBMCs), respectively (Figure 2*A*), and the predominating isotype was IgA in 6 of 14 volunteers and IgG in 8 of 14 volunteers (Figure 2*A*). In the PCV group, the respective median values were 82 ASCs/10^6 PBMCs (range, 1–212 ASCs/10^6 PBMCs), 109 ASCs/10^6 PBMCs (range, 19–626 ASCs/10^6 PBMCs), and 10 ASCs/10^6 PBMCs (range, 0–153 ASCs/10^6 PBMCs; Figure 2*B*), and the response was dominated by IgA-ASCs in 3 of 11 volunteers, by IgG-ASCs in 7 of 11, and by IgM-ASCs in 1 of 11 volunteers (Figure 2*B*).

Expression of $\alpha_4\beta_7$, L-selectin, and CLA on *S. pneumoniae*–Specific ASCs

The homing receptor expressions in the 3 study groups are shown in Figure 3. The mean proportion (±SD) of $\alpha_4\beta_7$ -expressing

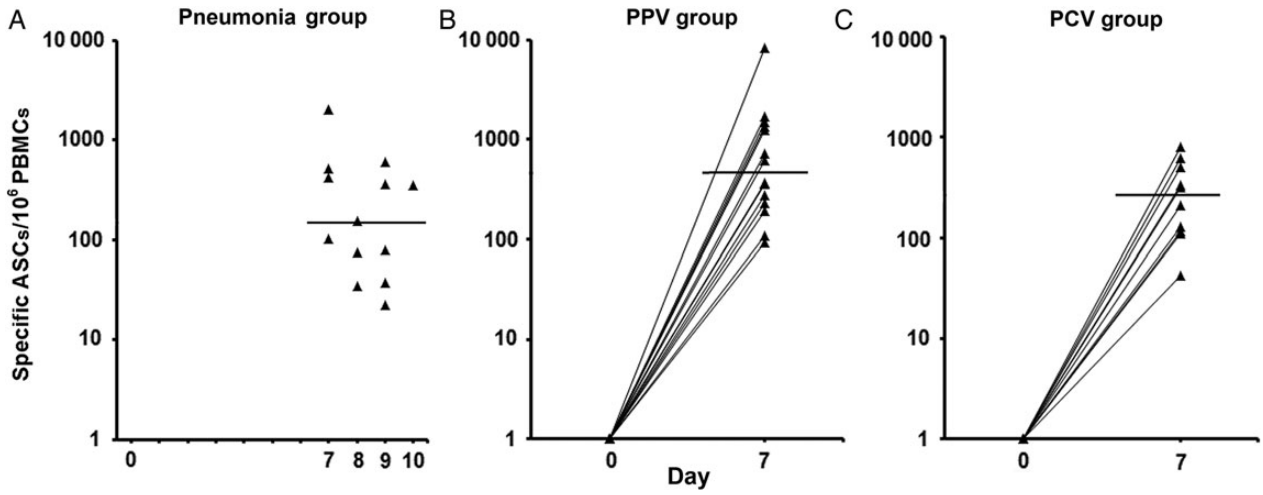

Figure 1. *Streptococcus pneumoniae*–specific plasmablast response in the pneumonia, pneumococcal polysaccharide vaccine (PPV), and pneumococcal conjugate vaccine (PCV) groups. *A*, Numbers of plasmablasts (antibody-secreting cells [ASCs])/10^6 peripheral blood mononuclear cells (PBMCs) specific to the *S. pneumoniae* isolate from each of the 16 patients in the acute phase of the disease (7–10 days after onset of symptoms). *B* and *C*, Numbers of *S. pneumoniae* (serotype 14 capsule polysaccharide)–specific ASCs/10^6 PBMCs on days 0 and 7 for 14 volunteers immunized with PPV (*B*) and for 11 volunteers immunized with PCV (*C*). The spots connected with a line indicate the ASC counts for individual subjects; the bars represent the medians.

cells among *S. pneumoniae*–specific ASCs was higher in the pneumonia group (44% ± 24%), compared with the vaccinated groups (10% ± 8% for the PPV group [$P < .0001$] and 16% ± 11% for the PCV group [$P = .029$]).

The majority of *S. pneumoniae*–specific ASCs expressed L-selectin in all the groups: the arithmetic mean proportions (±SD) of L-selectin–expressing cells were 79% ± 16%, 89% ± 9%, and 73% ± 20% for the pneumonia, PPV, and PCV groups,

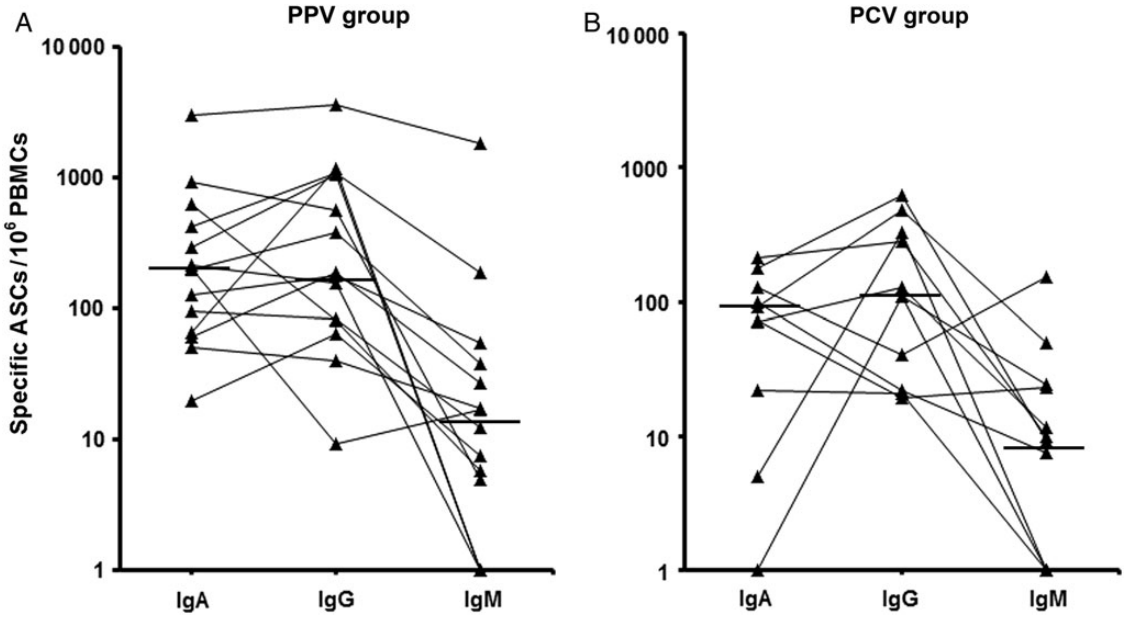

Figure 2. Isotype distribution of the *Streptococcus pneumoniae*–specific plasmablast response in the pneumococcal polysaccharide vaccine (PPV) and pneumococcal conjugate vaccine (PCV) groups. The numbers of *S. pneumoniae*–specific immunoglobulin A (IgA)–antibody-secreting cells (ASCs), immunoglobulin G (IgG)–ASCs, and immunoglobulin M (IgM)–ASCs per 10^6 peripheral blood mononuclear cells (PBMCs) on day 7 are presented for each individual, connected with a line. Data are shown separately for the PPV (n = 14; *A*) and PCV (n = 11; *B*) groups. The bars indicate the median value of each isotype. The respective data for the patients with pneumonia have been presented in our previous report [26].

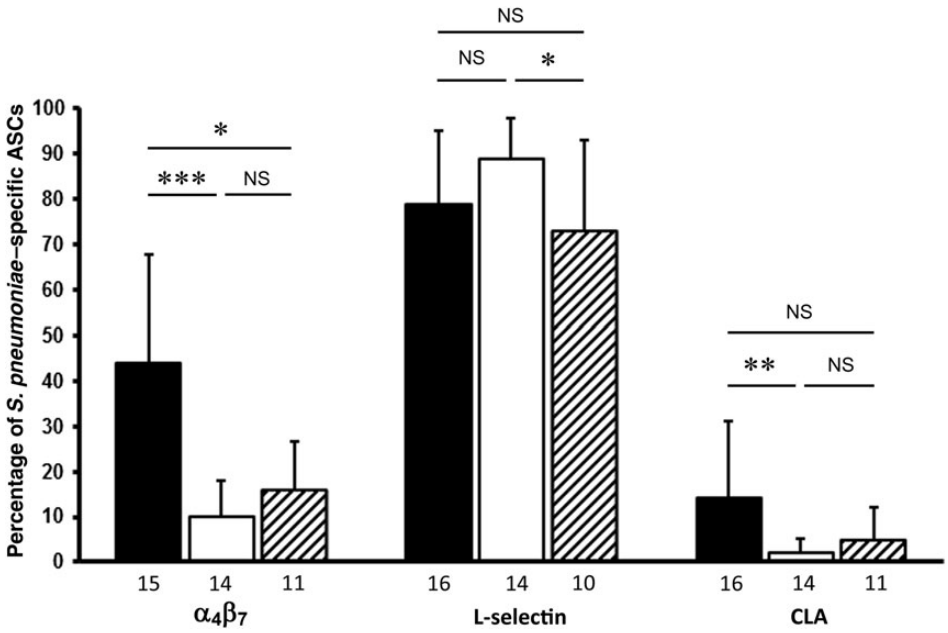

Figure 3. Expression of homing receptors on *Streptococcus pneumoniae*–specific plasmablasts in the 3 study groups. Expression of the homing receptors $\alpha_4\beta_7$, L-selectin, and cutaneous lymphocyte antigen (CLA) on circulating *S. pneumoniae*–specific plasmablasts (antibody-secreting cells [ASCs]) in patients with pneumococcal pneumonia (black bars) and volunteers immunized 7 days earlier with pneumococcal polysaccharide vaccine (white bars) or pneumococcal conjugate vaccine (hatched bars). The bars indicate arithmetic mean values (±SD) of percentages of homing receptor–positive plasmablasts among all *S. pneumoniae*–specific plasmablasts (immunoglobulin A+immunoglobulin G+immunoglobulin M). The numbers of cases included in the pooled data are indicated under the bars. ***$P < .001$, **$P < .01$, and *$P < .05$, by the Kruskal–Wallis test, followed by a post hoc test using the Dunn–Bonferroni method. Abbreviation: NS, not significant ($P \geq .05$).

respectively. The expression was more frequent in the PPV group, compared with the PCV group ($P = .014$) but did not differ between the pneumonia and the vaccination groups.

The mean proportion (±SD) of CLA-expressing cells among *S. pneumoniae*–specific ASCs was higher in the pneumonia group (14% ± 17%), compared with the PPV group (2% ± 3%; $P = .001$), while no difference was found between the pneumonia and the PCV groups (5% ± 7%; Figure 3).

The isotype distribution of the patients' *S. pneumoniae*–specific ASCs with a given homing receptor is provided in Figure 4.

Despite the nonidentical antigens used in the ELISPOT assay for patients with pneumonia (ie, the strain recovered from each patient) and vaccinees (purified *S. pneumoniae* polysaccharide), there was no indication of methodological reasons for the differences in homing receptor expressions. In a representative patient, the homing receptor expressions proved similar regardless of whether the patient's own *S. pneumoniae* strain or a mixture of *S. pneumoniae* polysaccharide antigens had been used in coating (26% and 25% for $\alpha_4\beta_7$, 85% and 87% for L-selectin, and 8% for CLA).

Expression of L-selectin, $\alpha_4\beta_7$, and CLA on All ISCs in the Pneumonia Group

The homing receptor profile of the *S. pneumoniae*–specific ISCs from patients with pneumonia is presented separately for each isotype in Figure 4.

DISCUSSION

Pneumococcal colonization is considered a prerequisite for *S. pneumoniae* pneumonia [1, 2]. However, carriage in itself is known to induce mucosal antibodies that may prevent colonization [3, 6–8, 33] and protect against pneumonia [34]. It has been suggested that aspirating or inhaling colonizing organisms during the first few weeks of colonization may lead to pneumonia, whereas after this period the individuals may be protected against the disease because of mucosal antibodies [34]. Consistent with the role of protective immune mechanisms induced by previous exposure, recurrences of pneumonia are rarely seen in immunocompetent individuals [35]. To design vaccines that elicit protective immune mechanisms closely mimicking those during an infection, in-depth information on natural immune responses is needed. The present study is the first to focus on the localization of LRT-originating B cells in a natural infection in humans. We explored the homing receptor expressions of newly activated plasmablasts in the circulation of patients with pneumonia. Significant differences in the homing profiles were revealed between patients with pneumonia and volunteers who received PPV or PCV.

The exact activation site in the human LRT is not known. Likewise, the homing receptors guiding lymphocytes to the LRT have not been fully identified, but a variety of them are

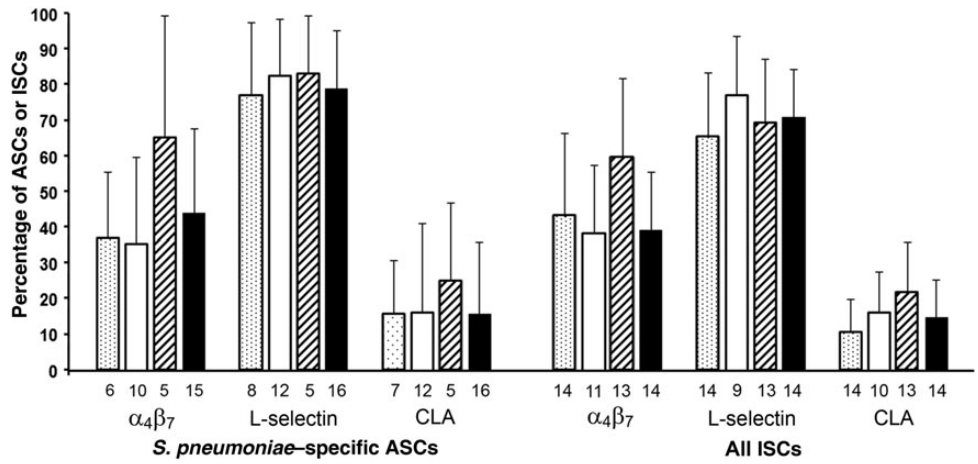

Figure 4. Expression of homing receptors on immunoglobulin A (IgA)–antibody-secreting cells (ASCs), immunoglobulin G (IgG)–ASCs, immunoglobulin M (IgM)–ASCs, and all immunoglobulin-secreting cells (ISCs) in patients with pneumonia. The bars show the arithmetic mean values (±SD) of percentages of $\alpha_4\beta_7$-expressing, L-selectin–expressing, and cutaneous lymphocyte antigen (CLA)–expressing plasmablasts among circulating *Streptococcus pneumoniae*–specific plasmablasts (ASCs) and the total of ISCs, given separately for IgA (dotted bars), IgG (white bars), and IgM (hatched bars) isotypes and their total (black bars). The numbers of cases included in the pooled data are indicated under the bars.

assumed to contribute to this migration [16, 17, 25, 36, 37]. While most studies on lung homing have been carried out with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep lung lymph have been shown to be $\alpha_4\beta_7^{low}$ and L-selectin^{low} [38]. Likewise, human lung T cells are $\alpha_4\beta_7^{low}$ [39, 40], L-selectin$^{-\text{ to low}}$ [39], and CLA$^-$ [40] or CLA$^{-\text{ to low}}$ [39], a profile different from that of cells migrating to the gut ($\alpha_4\beta_7^{high}$, L-selectin^{low}, and CLA$^{-\text{ to low}}$) and skin ($\alpha_4\beta_7^{low}$, L-selectin^{high}, and CLA^{high}) [39, 40].

The possible role of $\alpha_4\beta_7$, L-selectin, and CLA in plasmablast homing to the LRT remains unclear. In the light of T-cell studies, our results showing a moderate proportion of $\alpha_4\beta_7$-expressing B cells (44%) and a high frequency of L-selectin–expressing B cells (79%) were both somewhat unexpected. Bronchus-associated lymphoid tissue (BALT) found in patients with lung carcinoma appears to recruit both naive and memory/effector lymphocytes, with α_4-integrin and L-selectin expressed on nearly all B cells and less than half of T cells [36]. In the same study, BALT proved positive for the endothelial ligands PNAd (peripheral lymph node addressin) and VCAM-1 (vascular cell adhesion molecule-1), but not MAdCAM-1 (mucosal vascular addressin cell adhesion molecule-1). The great proportion of α_4-expressing B cells in BALT may imply the presence of either $\alpha_4\beta_1$-integrins or $\alpha_4\beta_7$-integrins, both of which can bind VCAM-1 [19, 41], a ligand much more common in human BALT than in peripheral lymph nodes and Peyer patches [25, 36]. Recently, $\alpha_4\beta_1$ has been shown to contribute substantially to lymphocyte recruitment to the human LRT in inflammation [35]. While T-cell homing to the LRT may not be mediated by $\alpha_4\beta_7$ [37, 39, 40], in mice, $\alpha_4\beta_7$/VCAM-1 interaction participates in the homing of mast cells [41]; the role of $\alpha_4\beta_7$ in B-cell homing to the LRT remains unclear.

While pneumonia is preceded by pneumococcal colonization of the URT, plasmablasts in the present study might, in fact, also have originated in the URT. However, in our ongoing study of patients with sinusitis, such a strong plasmablast response is only seen in an acute disease, not in prolonged cases. Furthermore, the homing receptor profile in acute URT infection differs from that presented here for patients with pneumonia (N. V. Palkola, unpublished data). Thus, we understood the homing receptors in the present study to reflect the destinations of LRT-originating circulating plasmablasts trafficking both to lung tissue and other potential mucosal sites: our data suggest that both L-selectin and $\alpha_4\beta_7$ contribute to distributing the humoral immune response elicited in the human LRT.

The first of the 2 fundamental findings of the present study was that, in pneumonia (in which antigen is encountered in the LRT), the homing profile differed from those reported previously in any of the other sites—after parenteral tetanus toxoid vaccination [42], oral [22, 23], rectal [23], and parenteral [22] typhoid vaccination, or intranasal [21] and rectal [21] cholera vaccination, as well as in mucosal infections in the intestine [29, 43] or urinary tract [42]. These studies have shown that cells activated in the intestine [21–23] all express $\alpha_4\beta_7$, while only a moderate proportion express L-selectin, and practically none express CLA and that intranasal immunization [21] elicits a response with a great proportion of $\alpha_4\beta_7$-expressing and L-selectin–expressing cells. Parenteral vaccination [22, 42], by contrast,

elicits a response in which the proportion of $\alpha_4\beta_7$-expressing cells is low, the proportion of L-selectin–expressing cells is high, and the proportion of CLA-expressing cells is moderate. The present study is the first to explore the homing receptor profile of plasmablasts originating in the LRT. It shows that plasmablasts in patients with pneumonia are characterized by a high proportion of L-selectin–expressing, moderate $\alpha_4\beta_7$-expressing, and low CLA-expressing cells. The unique homing receptor profile in pneumonia appears to agree with the general understanding that the localization of an immune response depends on the site of antigen encounter [23]. It also accords with mucosal route of antigen encounter being more potent than parenteral route in inducing mucosal response [3, 6, 7]. Interestingly, significant coexpression of various homing receptors was found, a phenomenon also seen after booster immunization in our previous research [44]. Consistent with the unique homing receptor profile of B cells in our study, the expression of known homing signals of human lung T cells has been found distinct from gut-homing and skin-homing T cells [39, 40].

The high expression of L-selectin detected in pneumonia agrees with reports of PNAd expression in the LRT [25, 36]. The role of $\alpha_4\beta_7$, on the other hand, deserves discussion. One of its 2 potential ligands, VCAM-1, is expressed in the lung tissue, while the main ligand, MAdCAM-1, is not [25, 41, 36]. The $\alpha_4\beta_7$ expression may reflect a number of points: (1) a role for $\alpha_4\beta_7$ in lymphocyte homing to the LRT, as discussed above; (2) a booster-type response to a previous encounter with *S. pneumoniae* in the URT or the intestine [44, 45]; or, most logically, (3) communication between mucosa-associated tissues in the body, with immunization at one mucosal site leading to an immune response at others [23], presumably serving to ensure protection at potential sites of future antigen encounter. Indeed, the main ligand for $\alpha_4\beta_7$, MAdCAM-1, has been shown to be expressed not only in the intestine but also in the URT [46]—the optimal location for an immune response serving to ward off bacterial pneumonia.

The second of our 2 major findings was that the homing receptor profile of plasmablasts in *S. pneumoniae* pneumonia differed from that after receipt of PPV and PCV. As for PPV, this was seen in 2 of the homing receptor types examined: in the patients with pneumonia the proportion of cells expressing $\alpha_4\beta_7$ and CLA was greater than in the PPV group. The homing receptor profile in the PCV group was found to be closer to that in patients with pneumonia, with merely 1 difference: $\alpha_4\beta_7$ was expressed more frequently in the pneumonia than the PCV group.

The high frequency of L-selectin–expressing plasmablasts in all study groups accords both with the role of L-selectin as a homing receptor guiding cells to the systemic immune system [18] and indications of its participation in B-cell homing to the lung [25, 36]. The more frequent expression of $\alpha_4\beta_7$ among patients with pneumonia than vaccinees, on the other hand, suggests a more mucosa-oriented homing from the LRT, discussed above. Intriguingly, poor immune response localization to mucosal sites, especially to the URT and/or LRT, as suggested by the low frequency of $\alpha_4\beta_7$-expressing cells after parenteral *S. pneumoniae* vaccination, might explain the shortcomings of *S. pneumoniae* vaccines in protecting against local disease. While both PPV and PCV appear to contribute to the local pulmonary immune response through serum-derived antibodies [47], PCV, unlike PPV, has also been shown to be able to reduce colonization by *S. pneumoniae* [48]. Furthermore, as yet another indication of local immunity, PCVs have proven to be efficacious against acute otitis media [49]. PCVs thus appear to contribute to mucosal immunity more than PPVs; consistently, the homing receptor profile in the PCV group was closer to that in the pneumonia group.

The limitations of this research include 2 points to be discussed: the selection of patients and the homing receptors chosen for examination. Only bacteremic patients with pneumonia could be recruited, because in them the individual *S. pneumoniae* isolate could be retrieved from blood culture and used as antigen. In these cases, however, even if the first pathogen encounter occurs in the respiratory tract, bacteremia may trigger some additional systemic stimulation. While this may skew the homing receptor profile in the pneumonia group toward a systemic rather than local type, it is remarkable that, compared with vaccinees, the homing receptor profile in pneumonia showed a trend toward a more mucosa-oriented type, even though only bacteremic patients had been included. While the present data do not allow comparisons between homing receptor profiles of bacteremic and nonbacteremic patients with pneumonia, in our previous study the homing receptor profile of subjects with urosepsis and nonbacteremic pyelonephritis was similar, and the bacteremia per se was not found to have any effect on the homing receptor expressions [42].

Because of the restricted number of PBMCs available, only 3 homing receptors could be explored. It would, however, have been interesting to examine other markers, as well. One of the reasons for selecting $\alpha_4\beta_7$, L-selectin, and CLA was that these have been studied in infections at other mucosal sites [29, 42, 43], enabling comparisons. Even if all potential markers guiding cells home to the LRT were not covered, the differences detected through these 3 suggest a unique homing receptor profile for plasmablasts activated in the human LRT.

In conclusion, 2 important novel aspects are revealed in this study: (1) circulating pathogen-specific plasmablasts in patients with pneumonia have a unique homing profile quite unlike that found at other mucosal sites, and (2) the homing receptor profile in patients with pneumonia is more mucosa oriented than that seen after administration of PPV or PCV yet closer to that in PCV recipients than in PPV recipients. Dissimilarities in the localization of immune response may contribute to the differing efficacy of these vaccines against noninvasive versus invasive *S. pneumoniae* diseases.

Notes

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References

1. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis **2004**; 4:144–54.
2. Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines **2012**; 11:841–55.
3. Twigg HL III. Humoral immune defense (antibodies): recent advances. Proc Am Thorac Soc **2005**; 2:417–21.
4. Goldblatt D, Hussain M, Andrews N, et al. Antibody responses to nasopharyngeal carriage of *Streptococcus pneumoniae* in adults: a longitudinal household study. J Infect Dis **2005**; 192:387–93.
5. Jambo KC, Sepako E, Heyderman RS, Gordon SB. Potential role for mucosally active vaccines against pneumococcal pneumonia. Trends Microbiol **2010**; 18:81–9.
6. Richards L, Ferreira DM, Miyaji EN, Andrew PW, Kadioglu A. The immunising effect of pneumococcal nasopharyngeal colonisation; protection against future colonisation and fatal invasive disease. Immunobiology **2010**; 215:251–63.
7. Sun K, Johansen F-E, Eckmann L, Metzger DW. An important role for polymeric Ig receptor-mediated transport of IgA in protection against *Streptococcus pneumoniae* nasopharyngeal carriage. J Immunol **2004**; 173:4576–81.
8. Ferreira DM, Darrieux M, Silva DA, et al. Characterization of protective mucosal and systemic immune responses elicited by pneumococcal surface protein PspA and PspC nasal vaccines against a respiratory pneumococcal challenge in mice. Clin Vaccine Immunol **2009**; 16:636–45.
9. Pitsiou GG, Kioumis IP. Pneumococcal vaccination in adults: Does it really work? Respir Med **2011**; 105:1776–83.
10. Moberley SA, Holden J, Tatham DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. Cochrane Database Syst Rev **2013**; 1:CD000422.
11. Ochoa-Gondar O, Vila-Corcoles A, Rodriguez-Blanco T, et al. Effectiveness of the 23-valent pneumococcal polysaccharide vaccine against community-acquired pneumonia in the general population aged ≥60 years: 3 years of follow-up in the CAPAMIS study. Clin Infect Dis **2014**; 58:909–17.
12. von Gottberg A, de Gouveia L, Tempia S, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med **2014**; 371:1889–99.
13. Cutts FT, Zaman SMA, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomized, double-blind, placebo-controlled trial. Lancet **2005**; 365:1139–46.
14. Lucero MG, Dulalia VE, Nillos LT, et al. Pneumococcal conjugate vaccines for preventing vaccine-type invasive pneumococcal disease and X-ray defined pneumonia in children less than two years of age. Cochrane Database Syst Rev **2009**; 4:CD004977.
15. Angoulvant F, Levy C, Grimprel E, et al. Early impact of 13-valent pneumococcal conjugate vaccine on community-acquired pneumonia in children. Clin Infect Dis **2014**; 58:918–24.
16. Brandtzaeg P, Johansen FE. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev **2005**; 206:32–63.
17. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. Nature Immunol **2008**; 9:981–7.
18. Camerini D, James SP, Stamenkovic I, Seed B. Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor. Nature **1989**; 342:78–82.
19. Berlin C, Berg EL, Briskin MJ, et al. α4β7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell **1993**; 74:185–95.17.
20. Berg EL, Yoshino T, Rott LS, et al. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. J Exp Med **1991**; 174:1461–6.
21. Quiding-Järbrink M, Nordström I, Granström G, et al. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. J Clin Invest **1997**; 99:1281–6.
22. Kantele A, Kantele JM, Savilahti E, et al. Homing potentials of circulating lymphocytes in humans depend on the site of activation: oral, but not parenteral, typhoid vaccination induces circulating antibody-secreting cells that all bear homing receptors directing them to the gut. J Immunol **1997**; 158:574–9.
23. Kantele A, Häkkinen M, Moldoveanu Z, et al. Differences in immune responses induced by oral and rectal immunizations with *Salmonella typhi* Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. Infect Immun **1998**; 66:5630–5.
24. Sundström P, Lundin SB, Nilsson L-Å, Quiding-Järbrink M. Human IgA-secreting cells induced by intestinal, but not systemic, immunization respond to CCL25 (TECK) and CCL28 (MEC). Eur J Immunol **2008**; 38:3327–38.
25. Xu B, Wagner N, Pham LN, et al. Lymphocyte homing to bronchus-associated lymphoid tissue (BALT) is mediated by L-selectin/PNAd, α4β1 integrin/VCAM-1, and LFA-1 adhesion pathways. J Exp Med **2003**; 197:1255–67.
26. Palkola NV, Pakkanen SH, Kantele JM, Rossi N, Puohimiemi R, Kantele A. Pathogen-specific circulating plasmablasts in patients with pneumonia. PLoS One **2012**; 7:e34334.
27. Kantele A, Arvilommi H, Jokinen I. Specific immunoglobulin-secreting human blood cells after peroral vaccination against *Salmonella typhi*. J Infect Dis **1986**; 153:1126–31.
28. Kantele A. Antibody-secreting cells in the evaluation of the immunogenicity of an oral vaccine. Vaccine **1990**; 8:321–6.
29. Kantele JM, Arvilommi H, Kontiainen S, et al. Mucosally activated circulating human B cells in diarrhea express homing receptors directing them back to the gut. Gastroenterology **1996**; 110:1061–7.
30. Kantele AM, Takanen R, Arvilommi H. Immune response to acute diarrhea seen as circulating antibody-secreting cells. J Infect Dis **1988**; 158:1011–6.
31. Kantele A, Zivny J, Häkkinen M, Elson CO, Mestecky J. Differential homing commitments of antigen-specific T cells after oral or parenteral immunization in humans. J Immunol **1999**; 162:5173–7.
32. Kantele J, Kantele A, Arvilommi H. Circulating immunoglobulin-secreting cells are heterogeneous in their expression of maturation markers and homing receptors. Clin Exp Immunol **1996**; 104:525–30.

33. Weinberger DM, Dagan R, Givon-Lavi N, et al. Epidemiologic evidence for serotype-specific acquired immunity to pneumococcal carriage. J Infect Dis **2008**; 197:1511–8.
34. Musher DM, Groover JE, Reichler MR, et al. Emergence of antibody to capsular polysaccharides of *Streptococcus pneumoniae* during outbreaks of pneumonia: association with nasopharyngeal colonization. Clin Infect Dis **1997**; 24:441–6.
35. McEllistrem MC, Mendelsohn AB, Pass MA, et al. Recurrent invasive pneumococcal disease in individuals with human immunodeficiency virus infection. J Infect Dis **2002**; 185:1364–8.
36. Kawamata N, Xu B, Nishijima H, et al. Expression of endothelia and lymphocyte adhesion molecules in bronchus-associated lymphoid tissue (BALT) in adult human lung. Respir Res **2009**; 10:97.
37. Walrath JR, Silver RF. The α4β1 integrin in localization of mycobacterium tuberculosis–specific T helper type 1 cells to the human lung. Am J Respir Cell Mol Biol **2011**; 45:24–30.
38. Abitorabi MA, Mackay CR, Jerome EH, Osorio O, Butcher EC, Erle DJ. Differential expression of homing molecules on recirculating lymphocytes from sheep gut, peripheral, and lung lymph. J Immunol **1996**; 156:3111–7.
39. Picker LJ, Martin RJ, Trumble A, et al. Differential expression of lymphocyte homing receptors by human memory/effector T cells in pulmonary versus cutaneous immune effector sites. Eur J Immunol **1994**; 24:1269–77.
40. Campbell JJ, Brightling CE, Symon FA, et al. Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. J Immunol **2001**; 166:2842–8.
41. Abonia JP, Hallgren J, Jones T, et al. Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. Blood **2006**; 108:1588–94.
42. Kantele AM, Palkola NV, Arvilommi HS, Kantele JM. Distinctive homing profile of pathogen-specific activated lymphocytes in human urinary tract infection. Clin Immunol **2008**; 128:427–34.
43. Pakkanen SH, Kantele JM, Moldoveanu Z, et al. Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. Clin Vaccine Immunol **2010**; 17:393–401.
44. Kantele A, Arvilommi H, Iikkanen K, et al. Unique characteristics of the intestinal immune system as an inductive site after antigen reencounter. J Infect Dis **2005**; 191:312–7.
45. Kantele A, Savilahti E, Tiimonen H, Iikkanen K, Autio S, Kantele JM. Cutaneous lymphocyte antigen expression on human effector B cells depends on the site and on the nature of antigen encounter. Eur J Immunol **2003**; 33:3275–83.
46. Csencsits KL, Jutila MA, Pascual DW. MadCAM-1 in NALT endothelial venules in a mucosal site in naive lymphocyte adhesion to high primary role of peripheral node addressin phenotypic and functional evidence for the nasal-associated lymphoid tissue. J Immunol **1999**; 163:1382–9.
47. Gordon SB, Miller DE, Day RB, et al. Pulmonary immunoglobulin responses to *Streptococcus pneumoniae* are altered but not reduced in human immunodeficiency virus–infected Malawian adults. J Infect Dis **2003**; 188:666–70.
48. Käyhty H, Auranen K, Nohynek H, Dagan R, Mäkelä PH; the Pneumococcal Carriage Group (PneumoCarr). Nasopharyngeal colonization: a target for pneumococcal vaccination. Expert Rev Vaccines **2006**; 5: 651–68.
49. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med **2001**; 344:403–9.