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

Nina V. Palkola,1,2 Sari H. Pakkanen,1 Jussi M. Kantele,5 Laura Pakarinen,2 Ritvaleena Puohiniemi,4 and Anu Kantele2,3

1Department of Bacteriology and Immunology, 2Department of Clinical Medicine, University of Helsinki, 3Inflammation Center, Clinic of Infectious Diseases, 4Department of Clinical Microbiology, HUSLAB, Helsinki University Hospital and University of Helsinki, and 5Department of Medical Microbiology and Immunology, University of Turku, Finland

**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 α4β7, 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 α4β7 was moderate, and the proportion expressing CLA was low. The homing receptor α4β7 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.

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 (CCR) 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], α4β7-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 CRM197 protein and 0.004 mg of serotype 6B conjugated with CRM197 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 anti human α4β7 (ACT-1), anti human L-selectin (Leu 8), or anti human 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 1A), 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 1B and 1C). 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 1B) and 265 ASCs/10^6 PBMCs (range, 43–812 ASCs/10^6 PBMCs) in the PCV group (Figure 1C).

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 2A), and the predominating isotype was IgA in 6 of 14 volunteers and IgG in 8 of 14 volunteers (Figure 2A). 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 2B), 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 2B).

**Expression of α4β7, 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 α4β7-expressing
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, respectively.

![Figure 1](https://example.com/image1.png)

**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.

![Figure 2](https://example.com/image2.png)

**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].
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
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 with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector/memory T cells in sheep with T cells, our study explores homing receptor expression on B cells originating in the LRT. Effector memoria
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, bacteria 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

Acknowledgments. We thank the personnel of the Department of Clinical Microbiology, HUSLAB, Helsinki University Hospital, for help in providing the bacterial strains; and Helena Käyhty, from the National Institute for Health and Welfare (Helsinki), for providing the pneumococcal polysaccharide for the analyses.

Disclaimer. The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Financial support. This work was supported by the Finnish Medical Association (to A. K., N. V. P., and S. H. P.), the Finnish governmental subsidy for health science research (to A. K. and S. H. P.), the Finnish Tuberculosis Foundation (to N. V. P.), the Research Foundation of the Pulmonary Diseases (to N. V. P.), the Foundation of Emil Aaltonen (to N. V. P.), and the Paulo Foundation (to A. K.).

Potential conflicts of interest. A. K. has participated as a member in advisory boards of Pfizer, GlaxoSmithKline, and Novartis; has received honoraria for lectures given for Pfizer and GlaxoSmithKline; and has received an investigator-initiated research grant from Pfizer. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Bogaert D, De Groot R, Hermans PW. Streptococcus pneumoniae colonization: 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. Jamo 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, Kiousis 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:CD004222.
11. Ochoa-Gonzald O, Vila-Concelos 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, Emwere 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, Dulala 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. Brandtzæg 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-7/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. Quinding-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, Håkkinen 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, Quinding-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, Puhkimieri 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, Konttinen 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 diarrhoea seen as circulating antibody-secreting cells. J Infect Dis 1988; 158:1011–6.
31. Kantele A, Zivny J, Håkkinen 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. 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, Ilkkanen 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, Saviáhti E, Tiimonen H, Ilkkanen 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 naïve 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.