Expression of Homing Receptors on IgA1 and IgA2 Plasmablasts in Blood Reflects Differential Distribution of IgA1 and IgA2 in Various Body Fluids

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Although secretory IgA is the most abundantly produced Ig isotype, the mechanisms underlying the differential distribution of IgA subclasses in various body fluids remain unclear. To explore these mechanisms, we examined the distribution of IgA subclasses, the influence of the nature and sites of encounters with antigens, and the correlation between IgA subclass distribution and homing potentials of circulating IgA plasmablasts. IgA1 predominated in serum, tears, nasal wash fluid, and saliva; the levels of IgA1 and IgA2 were comparable in vaginal wash fluid; and IgA2 predominated in intestinal lavage fluids. Seventy-one percent of circulating IgA plasmablasts secreted IgA1. The intestinal homing receptor (HR), α4β7, was expressed more frequently on IgA2 than on IgA1 plasmablasts, with no differences in the expression of other HRs. IgA subclass distribution among circulating antigen-specific antibody-secreting cells (ASC) was dependent on the nature of the antigen: following vaccination with Salmonella enterica serovar Typhi, unconjugated pneumococcal polysaccharide, or Haemophilus influenzae polysaccharide-diphtheria toxoid conjugate, the proportions of specific IgA1 ASC were 74%, 47%, 56%, and 80%, respectively. HR expression depended on the route of administration: expression of HRs was different after oral than after parenteral vaccination, while no difference was seen between HR expression of antigen-specific IgA1 and IgA2 ASC induced via the same route. The key factors determining IgA subclass distribution in a given secretion are the nature of the antigens encountered at a particular site and the site-specific homing instructions given to lymphocytes at that site. These two factors are reflected as differences in the homing profiles of the total populations of circulating IgA1 and IgA2 plasmablasts.

Because humoral immunity is most frequently evaluated by titers of serum antibodies, which are dominated by IgG, the role of IgA as the major Ig isotype produced in humans has not been adequately appreciated: the daily production of IgA (66 mg/kg of body weight/day) exceeds that of all other immunoglobulin classes combined (10, 14, 40). Even if IgA levels in serum are lower than those of IgG, due to a shorter half-life in the circulation, IgA is the predominant immunoglobulin in most external secretions (3, 14, 40). IgA, as the mucosal Ig isotype, plays a dominant role as the first immunological defense barrier in the body, since the mucosal sites act as a portal of entry to the majority of human pathogens (3, 40, 53).

In humans, IgA comprises two subclasses, IgA1 and IgA2, which are unequally distributed in the body fluids (4, 13, 34, 36, 41). In serum, IgA1 is dominant, while in secretions, there is a significant contribution of IgA2. In external secretions, both IgA1 and IgA2 are present as secretory IgA (S-IgA), a polymeric form which is more resistant to proteolytic enzymes than any of the other isotypes (40). S-IgA is known to provide protection of mucosal membranes in several complementary ways: it can effectively neutralize viruses (46, 47, 49) or toxins (47) and displays antibacterial activity (47, 51). To evade the protective effect of S-IgA at the mucosal sites, some pathogenic bacteria (e.g., Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, and Neisseria gonorrhoeae) produce proteases which cleave the IgA1 molecule (31), leaving the IgA2 intact. The unequal distribution of IgA subclasses in different body fluids (5, 13, 28) and among IgA-secreting cells in the circulation (25) and mucosal and systemic lymphoid tissues (11, 30) has been reported in several independent studies. The differential distribution of the two IgA subclasses in secretions has been shown to be accompanied by a similar distribution of IgA1- and IgA2-producing cells at those sites (11, 30). IgA1 cells predominate in the bone marrow, and they are also present in higher proportions in upper parts of the oroantral and the respiratory tracts, while IgA2 cells predominate in the lower part of the gastrointestinal tract (11, 30). The basis for this differential distribution of IgA subclasses in various mucosal sites is not understood.

More than two-thirds of all Ig-producing cells in the body reside in the various mucosal and exocrine sites (40), particularly along the gastrointestinal tract, and most of them produce IgA specific for antigens encountered at mucosal surfaces. The major source of the precursors of the intestinal IgA plasma cells is the naive B cells in the organized lymphoepithelial tissues, such as the Peyer’s patches (12). B cells in the germinal
centers of Peyer's patches switch from IgM+ to IgA+ under the influence of resident cells and various cytokines (8, 14, 38). Subsequently, the cells migrate to the draining mesenteric lymph node, where they continue to divide and differentiate into plasmablasts (12, 15, 16). Finally, they exit the lymph nodes and migrate via thoracic duct lymph into the blood, which carries them to the target tissues, such as the lamina propria of the gut, to secrete IgA. The homing of these activated lymphocytes from the circulation into mucosal tissues involves multiple steps (33, 43, 48) in which the two central events are (i) the interactions between the chemokine receptors (CCR) on the lymphocyte surface and the chemokines produced in the tissue and (ii) the recognition by the lymphocyte surface homing receptors (HR) of their tissue-specific ligands, called addressins, on the surface of the endothelial cells in the mucosa. The key homing receptor-addressin interaction in lymphocyte homing to the intestinal tissue is believed to be the binding of the gut HR, α4β7, on the lymphocyte surface to its ligand, MAdCAM-1, on the high endothelial venules of the intestinal lamina propria (2). A fraction of IgA+ plasmablasts in the circulation expresses α4β7 (21, 27), consistent with their ability to home to intestinal lamina propria. Some IgA plasmablasts express other HR (20–22, 26), such as the peripheral lymph node HR, t-selectin (6, 32), and the skin HR, cutaneous lymphocyte-associated antigen (CLA) (44), consistent with the ability of a portion of IgA plasmablasts to home to extraintestinal lymphoid tissues at systemic and mucosal sites (48). The HR guiding the cells to the different extraintestinal mucosal sites have not been identified, but it has been suggested that there would be several different HR and CCR contributing to the selective homing to extraintestinal mucosal sites (4, 54). In humans, it is possible to determine the expression of HR on circulating plasmablasts and interpret these data as homing potentials or homing profiles of these cells (22, 26, 27, 45). Furthermore, it might be assumed that the differential distribution of IgA1 and IgA2 plasma cells in the various sites in the body would depend on a differential homing potential of circulating IgA1 and IgA2 plasmablasts and, thus, could be predicted by the differential expression of HR.

Although there are data concerning the differential distribution of IgA subclasses in various body fluids (5, 9, 13, 34, 36, 41, 42), studies involving parallel distribution of IgA subclasses in various body fluids and circulating plasmablasts expressing mucosal and/or systemic HR are not available. In the present study, we (i) determined the differential distribution of IgA subclasses in various body fluids using identical assays and reagents, (ii) evaluated the factors influencing IgA subclass distribution of a specific immune response, and (iii) explored whether the differential distribution of IgA subclasses in the various body fluids is associated with differential homing profiles of IgA1 and IgA2 plasmablasts in the circulation.

MATERIALS AND METHODS

In the present study, we investigated two types of cells in the circulation: all immunoglobulin-secreting cells (ISC) and antigen-specific antibody-secreting cells (ASC). All ISC represent only a small fraction of all circulating B cells, the fraction which has developed into the effector-cell stage, actively secretes immunoglobulins, and is known as plasmablasts. These ISC represent the sum of numerous ASC populations, each specific to different antigens encountered recently by the individual. In the present study, antigen-specific ASC are a population of plasmablasts producing specific antibodies against the specific vaccine antigen with which the volunteer was vaccinated 7 days earlier.

Study design. A total of 73 healthy volunteers were enrolled in the study; 61 were females and 12 males, aged 21 to 48 years. None of the participants was IgA deficient. Fifty-five volunteers provided blood samples, and 16 provided both secretions and blood samples. Fifty-three volunteers with no history of the vaccination or the respective disease were vaccinated and assessed for vaccine antigen-specific responses in their secretions, sera, or whole blood (B cells). The vaccinations and sample collection were performed after written informed consent. The study protocol was approved by the Ethics Committees of the University of Alabama at Birmingham and of the Helsinki University Central Hospital.

Sixteen women were enrolled for collection of external secretions (tears, parotid saliva, nasal wash fluid, intestinal lavage fluid, and cervicovaginal secretions), serum, and heparinized whole blood, all collected during one day. They all had regular menstrual cycles; two were on birth control pills. Twelve volunteers were a subpopulation of a previous study (20) who had been vaccinated orally (7) or rectally (5) with Salmonella enterica serovar Typhi Ty21a and had provided samples of external secretions (tears, parotid saliva, nasal wash fluid, intestinal lavage fluid, and cervicovaginal secretions), serum, and heparinized whole blood, all collected during one day. They all had regular menstrual cycles; two were on birth control pills. Twelve volunteers were a subpopulation of a previous study (20) who had been vaccinated orally (7) or rectally (5) with Salmonella enterica serovar Typhi Ty21a and had provided samples of external secretions (tears, parotid saliva, nasal wash fluid, intestinal lavage fluid, and cervicovaginal secretions), serum, and heparinized whole blood, all collected during one day. They all had regular menstrual cycles; two were on birth control pills. Twelve volunteers were a subpopulation of a previous study (20) who had been vaccinated orally (7) or rectally (5) with Salmonella enterica serovar Typhi Ty21a and had provided samples of external secretions (tears, parotid saliva, nasal wash fluid, intestinal lavage fluid, and cervicovaginal secretions), serum, and heparinized whole blood, all collected during one day. They all had regular menstrual cycles; two were on birth control pills.

Vaccinations. Twelve volunteers were given typhoid vaccine orally, 11 rectally, and seven parenterally. Ten volunteers were given the Haemophilus influenzae type b (Hib-D) conjugate vaccine parenterally, and 15 the unconjugated pneumococcal polysaccharide vaccine parenterally. The oral vaccine was the live, attenuated Salmonella serovar Typhi Ty21a vaccine (Vivotif; Crucell, Bern, Switzerland) administered as three doses two days apart orally, according to the manufacturer’s instructions, or rectally, as described earlier (20). The parenteral Hib-D vaccine was prepared by the Swiss Serum and Vaccine Institute (currently Crucell) according to a previously described protocol (19). The vaccine contained 0.5 × 109 formalin-killed bacteria per dose. The Hib-D vaccine was a conjugate vaccine (HibTITER; Lederle-Praxis Biologicals, Inc., United States) with a polysaccharide component (polysorbol phosphate [PRP]; 10 μg) conjugated to diphtheria toxoid (25 μg CRM197 protein). Pneumovax is a 23-valent commercial pneumococcal polysaccharide vaccine (Pasteur Mérieux, Lyon, France) which contains, per dose, 0.025 mg of capsular polysaccharide of each of the following serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. All three parenteral vaccines were administered as an intramuscular injection of 0.5 ml in the left arm.

Collection of external secretions and blood samples. The collection of all secretions has been described in detail earlier (20). Tears were collected with a capillary tube from the medial corner of the eye after stimulation with mist expressed from an orange peel. Nasal wash fluid was obtained by instillation of prewarmed saline in the nostrils and expelling after 30 s. Unstimulated parotid saliva was collected using a Schaefer cup placed over the parotid duct and centrifuged to remove debris. Intestinal secretions were obtained either with rectal wick (Polyfiltronics, Inc., Rockland, MA) or by the oral administration of Golytely (buffered polyethylene glycol solution; Braintree Laboratories, Braintree, MA) as 2 liters taken within 1 h. The effluent was collected and filtered through cheesecloth, protease inhibitors were added, and the fluid was clarified by centrifugation (20). The clarified supernatant fluid was collected and stored at −70°C until assay. The elution of proteins from the rectal wick samples was performed as described earlier (20). Cervicovaginal secretions were collected by instillation of saline into the vagina, rinsing, and aspiration with a plastic pipette, and samples were further centrifuged to remove particulate material. After the
addition of protease inhibitors, samples were divided into multiple aliquots. Sera and the external secretions were frozen immediately after collection and kept at −70°C until assay.

Assay of IgA1 and IgA2 concentrations in the body fluids. Samples were assayed for concentrations of total IgA1 and IgA2 by ELISA as described previously (17, 42). Briefly, ELISA plates (Nunc Maxisorp; Nunc, Roskilde, Denmark) were coated with human Ig-absorbed goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL), followed by overnight incubation with either anti-human IgA (Sigma) or anti-human IgA2 (Recognition Sciences Ltd., Birmingham, United Kingdom). Duplicate serial dilutions of the sample or standard were incubated overnight. Bound IgAs were detected with peroxidase-conjugated goat anti-human IgA (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). As substrate, o-phenylenediamine-H2O2 in citrate-phosphate buffer (pH 5.0) was used; development was stopped with 1 M sulfuric acid after 15 min, and absorbance was read at 490 nm in a Vmax microplate reader (Molecular Devices Corp., Menlo Park, CA).

Assay of the concentrations of Salmonella serovar Typhi-specific IgA1 and IgA2 in the body fluids. The volunteers whose Salmonella serovar Typhi-specific IgA1 and IgA2 concentrations in body fluids were determined were a subgroup from another study (20). These samples were assayed for concentrations of total and Salmonella serovar Typhi-specific IgA1 and IgA2 by ELISA as described previously (42). Briefly, a whole-cell preparation of Salmonella serovar Typhi Ty21a was used for capture, the plates were blocked with 5% fetal bovine serum in phosphate-buffered saline (PBS)-Tween, and serial 2-fold dilutions of samples with ExtrAvidin peroxidase conjugate (Sigma-Aldrich). The wells were developed with o-phenylenediamine-H2O2 substrate (Sigma-Aldrich), the color reaction stopped with 1 M sulfuric acid, and the absorbance at 490 nm read.

Isolation of mononuclear cells. The mononuclear cells were isolated from the heparinized venous blood by Ficoll-Hypaque density gradient centrifugation as previously described (43).

Detection of the receptor-negative and -positive cell populations. The separation of the cells into receptor-negative and -positive populations has been described in detail previously (21, 24, 27). Briefly, cells were incubated with one of the following first-stage monoclonal antibodies: anti-α4β7 (ACT-1; Millennium Pharmaceuticals, Cambridge, MA), anti-β2-integrin (anti-CD11b; Becton-Dickinson, San Jose, CA), anti-α4 (anti-CD49d; Immunotech, Marseille, France), anti-α5β1 (anti-CD29 and anti-αVβ1; Immunotech), anti-α4β7, HLA-DR (Dako, Glostrup, Denmark), anti-CD28 (Becton-Dickinson), and anti-CD4 (BD Biosciences, San Jose, CA) in PBS, and the cells were incubated in the wells at four different concentrations, consistent with our previous results for Salmonella serovar Typhi-specific IgA1 and IgA2 concentrations. In the intestinal lavage fluid, IgA2 predominated (P < 0.001). As seen by the results in Fig. 1, there was a marked variability in levels of IgA1 and IgA2 among individuals.

Distribution of Salmonella serovar Typhi-specific IgA subclasses in various body fluids. The total and Salmonella serovar Typhi-specific IgA1 and IgA2 subclass distributions in body fluids collected from 12 volunteers were determined (20) (Table 3). Only in the intestinal lavage fluid was the total proportion of IgA2 higher than that of IgA1 (Table 1). Salmonella serovar Typhi-specific IgA1 and IgA2 were found in all secretions, consistent with our previous results for Salmonella serovar Typhi-specific total IgA (20). The concentration of Salmonella serovar Typhi-specific IgA1 exceeded that of Salmonella serovar Typhi-specific IgA2, consistent with the findings of the IgA1 subclass distribution of Salmonella serovar Typhi-specific IgA1 and IgA2 ASC in the blood (see below).

In the circulation of the vaccinees. Table 3 shows the numbers of IgA1- and IgA2-secreting cells specific for vaccine antigens in the blood on day 7 as a response to oral, rectal, or parenteral typhoid immunization or parenteral vaccination with the H. influenzae conjugate vaccine or pneumococcal polysaccharide vaccine.

The expression of homing receptors on IgA1 and IgA2 ASC. The frequencies of cells expressing α4β7, β2-integrin, and CLA among circulating IgA1 and IgA2 ASC are shown in Fig. 2. IgA2 ASC were found to express α4β7 significantly more frequently than IgA1 ASC, whereas no difference was found in the levels of expression of β2-integrin or CLA between the cells of the two subclasses. The levels of expression of α4β7, α5β1, α4β7-integrin, HLA-DR, and CD28 on IgA1 and IgA2 ASC are shown in Fig. 3. The statistical analysis did not reveal
any significant differences between the two subclasses with respect to the expression of any of these markers. However, a significant difference between IgA1 and IgA2 ISC was seen when the proportion of integrin-expressing cells was subtracted from the proportion of chain-expressing cells (Fig. 3). As chain is known to pair either with or chain, this approach has been used previously to describe the expression of when the monoclonal antibody to was not yet available.

The expression of homing receptors on vaccine antigen-specific IgA1 and IgA2 ASC after oral or parenteral vaccination. The total populations of IgA1 and IgA2 ISC represent the sum of smaller, antigen-specific populations of IgA1 and IgA2 ASC. Since there was a significant difference in the frequency

| Type of secretion | Salmonella serovar Typhi-specific IgA1/IgA2 | Total IgA1/IgA2 |
|-------------------|---------------------------------------------|-----------------|
| Saliva            | 5.3 ± 3.9                                   | 1.9 ± 3.0       |
| Nasal wash fluid  | 6.8 ± 4.6                                   | 2.1 ± 1.2       |
| Rectal-wick-sampled mucus | 2.9 ± 1.1                            | 0.4 ± 0.2       |
| Tears             | 3.9 ± 2.8                                   | 1.1 ± 0.4       |

* Data for orally and rectally immunized volunteers (n = 12) are pooled together.
of α4β7-expressing cells among the total populations of circulating IgA1 and IgA2 ISC (see above), it was of interest to examine the causes of this difference by focusing on small antigen-specific populations of ASC. The expression levels of HR were compared between antigen-specific IgA1 and IgA2 ASC in the peripheral blood of seven volunteers vaccinated orally with *Salmonella* serovar Typhi Ty21a and in five volunteers after parenteral pneumococcal polysaccharide vaccination. The HR data are only given for those volunteers whose ASC numbers reached the inclusion limit of 20 ASC detected (see Materials and Methods). Consistent with our earlier results (23), the frequencies of cells expressing the different HR differed between the two vaccination groups (Fig. 4): after oral Ty21a vaccination, all antigen-specific ASC expressed α4β7 only and a small amount expressed L-selectin, indicating an intestinal homing profile of these cells, while after pneumococcal vaccination, the frequency of α4β7 ASC was low and that of L-selectin ASC high, consistent with the systemic profile of HR expression. Notably, a comparison of HR expression on antigen-specific IgA1 and IgA2 ASC after an oral Ty21a or intramuscular pneumococcal vaccination revealed no differences between their frequencies on IgA1 and IgA2 ASC, indicating that both IgA1 and IgA2 cells antigen sensitized on a given site (mucosal versus systemic) have a similar homing potential.

### DISCUSSION

The present study confirms and extends the earlier notions of the differential distribution of IgA subclass antibodies in various body fluids (13). A significantly higher proportion of IgA1 than of IgA2 was found in serum, tears, nasal wash fluid, and saliva, while no difference was seen in the distribution in the vaginal wash fluid, and in the intestinal lavage fluid, IgA2 predominated. The proportions of IgA1+ and IgA2+ cells in a given mucosal tissue approximate the IgA1 to IgA2 ratio in a corresponding secretion (11, 30). Unfortunately, precise comparative studies of the proportions of IgA1 and IgA2 ISC and the proportions of IgA1 and IgA2 in the corresponding secretion have not been performed with the use of identical subclass-specific reagents. In the present study, the only mucosal site with actual IgA2 predominance was the intestine. This is consistent with previous reports on the distribution of IgA1 and IgA2 cells at different mucosal sites: the mucosa of the

### TABLE 2. Numbers of ISC isotypes in the peripheral blood of unimmunized healthy volunteers

| ISC isotype | No. of ISC/10^6 PBMC (geometric mean ± SEM) | % of IgA1-ISC |
|------------|------------------------------------------|---------------|
| IgG        |                                          |               |
| IgM        |                                          |               |
| IgA1       |                                          |               |
| IgA2       |                                          |               |
| IgA1 + IgA2|                                          |               |

*Data from 24 volunteers were pooled.*

*The percentage of ISC secreting IgA1 among all ISC was 73 ± 13 (mean ± standard deviation).*

### TABLE 3. Numbers of vaccine antigen-specific IgA1- and IgA2-secreting cells in the peripheral blood of healthy volunteers immunized with different vaccine regimens

| Vaccine regimen | Antigen specificity of ASC | No. (geometric mean ± SEM) of ASC/10^6 PBMC secreting: | % (arithmetic mean) of IgA1-secreting cells that secreted IgA1 | No. of volunteers | P value (paired t test) for IgA1 vs IgA2 |
|-----------------|----------------------------|-------------------------------------------------------|------------------------------------------------------------|------------------|-----------------------------------------|
| Oral Ty21a      | *Salmonella* serovar Typhi-specific cells | 69 ± 40 | 18 ± 27 | 84 ± 59 | 74 | 12 | <0.05 |
| Rectal Ty21a    | *Salmonella* serovar Typhi-specific cells | 21 ± 20 | 8 ± 5 | 27 ± 24 | 76 | 11 | <0.06 |
| Parenteral Ty21a| *Salmonella* serovar Typhi-specific cells | 15 ± 3 | 8 ± 3 | 24 ± 6 | 64 | 7 | <0.01 |
| Parenteral Hib-D| Diphtheria toxoid-specific cells | 856 ± 587 | 656 ± 310 | 1,600 ± 621 | 56 | 10 | NS |
| Parenteral Pnc   | Pnc polysaccharide type 14-specific cells | 129 ± 41 | 60 ± 19 | 164 ± 40 | 80 | 10 | <0.01 |

*Vaccine regimens included *Salmonella* serovar Typhi Ty21a given orally, rectally, or parenterally and *Haemophilus influenzae*-diphtheria toxoid conjugate vaccine or unconjugated pneumococcal polysaccharide (Pnc) vaccine given parenterally.*

*NS, difference was not significant.*
Colon has been shown to be the only mucosa with a pronounced dominance of IgA2 ISC (11, 29, 30). In previous studies, IgA1 and IgA2 cells were found in equal proportions in the female genital tract tissues (35) and the two subclasses were represented equally in the cervical mucus (34). The present data, with equal IgA1 and IgA2 concentrations in the vaginal wash fluid, confirm the data of those reports. The results of the present study concerning the distribution of IgA1 (60%) and IgA2 (40%) in tears are consistent with those of previous works by Delacroix et al. (13), Crago et al. (11), and Allansmith et al. (1) but differ from the results of Kett et al. (29). In the lacrimal glands, Crago et al. (11) found IgA1 and IgA2 cells in a ratio of 53:48 and Allansmith et al. (1) found them in a ratio of 56:44, consistent with the distribution of IgA1 and IgA2 in tears in the present study. In contrast, Kett et al. (30) found a more pronounced dominance of IgA1 cells (81%) in the lacrimal gland.

The mechanisms underlying the selective distribution of IgA1 and IgA2 and, particularly, the preponderance of IgA2 in the large intestine are not fully understood. Tarkowski et al. (50) and Lue et al. (37) have shown that polysaccharide vaccines elicit a dominant IgA2 response both in serum and in ASC. These results are consistent with those of the present study: an IgA2-dominated ASC response to a polysaccharide antigen from pneumococci was found, while an IgA1-dominated response was elicited to a protein antigen, diphtheria toxoid. Interestingly, a clear IgA2 dominance was not found in the response to the PRP antigen of Haemophilus influenzae, which could be associated with the type of vaccine: this vaccine is a protein conjugate vaccine, where the polysaccharide antigen is linked to a protein carrier, which alters the responses to T-cell-dependent type (39). Responses to Ty21a were measured against a whole-cell preparation of bacteria. Even if the O-9,12 polysaccharide antigen is probably the major antigen against which the response is mounted, some protein antigens on the surface of the bacteria used for coating may be a target of minor responses as well (19) and shift the response toward IgA1, as suggested by the data in Table 3. Findings of the distribution of IgA subclass response as related to the type of antigen suggest that the IgA2 dominance in the large intestine could be a consequence of a significant local stimulation with predominantly polysaccharide antigens and LPS (5, 7, 38), which would lead to the expansion of cells of the IgA2 subclass at this site. Consistently, the large intestine, with the most intensive local stimulation by endotoxin, displays more IgA2 than IgA1 cells (11, 28, 30). In the present study, the only other mucosal site where IgA2 reached the concentration of IgA1 was the vaginal fluid; the female genital tract is colonized by a significant Gram-negative bacterial population. In other mucosal sites, with less significant supplies of LPS, IgA1 cells have been shown to be more numerous than IgA2 cells (11, 30), and, in the present study also, IgA1 predominated in those secretions. The high proportion of IgA2 in the lower digestive tract and the female genital tract may be of functional importance: IgA2 is resistant to IgA1 proteases produced by some pathogenic bacteria (31), and moreover, IgA2 is more efficient than IgA1 in inhibiting bacterial adherence to the mucosal epithelium (52).

In order to search for the basis of differential distribution of IgA1 and IgA2 cells in the body, it appeared especially interesting to examine the HR expression on peripheral blood IgA1 and IgA2 plasmablasts before their potential homing to the systemic or mucosal tissues (4, 33, 48). It is probable that the differential distribution of IgA1 and IgA2 cells in various tissues would be a consequence of differential expression of HR and CCR on these cells in the circulation. Consistent with the highest proportions of IgA2 being found in intestinal lavage fluid, the intestinal HR, α4β7, was expressed more frequently on IgA2 than on IgA1 plasmablasts. The differential expression of HR on circulating IgA1 and IgA2 plasmablasts suggests that this is a key factor directing the cells of the two IgA subclasses to be differentially distributed in various tissues.

In addition to ISC, we examined the expression of HR on antigen-specific ASC after vaccination through mucosal and systemic routes with T-cell-dependent and -independent vaccines. The results confirmed our previous findings (21) that the expression of HR depends on the administration route: all orally induced ASC exhibited an “intestinal” HR profile, with all cells expressing α4β7 and a smaller proportion l-selectin, but after parenteral antigen administration, a systemic profile was seen, with only a small proportion of α4β7 cells and a higher proportion of l-selectin cells. Importantly,
the present study was the first to analyze HR on antigen-specific IgA1 and IgA2 ASC: IgA1 and IgA2 cells activated at the same site had similar HR profiles, indicating that the site of antigen encounter determines the HR expression of the two cell types.

It is of interest to consider why the frequency of α4β7-expressing cells was higher among IgA2 than IgA1 ISC. Because ISC in the blood represent the sum of numerous populations of ASC, each specific to some recently encountered antigen, and the intestine is known to be the only site of antigen encounter that results in 100% expression of α4β7, the high level of α4β7 expression on IgA2 ISC implies a high content of populations activated in the intestine. It seems reasonable to think that the high intestinal polysaccharide and LPS loads would result in high numbers of IgA2-dominated responses and, thus, shift the α4β7+ ISC among circulating IgA2 to a larger proportion than in IgA1 ISC. This also appears reasonable from the perspective that the majority of all plasma cells are known to be located at the intestinal sites (14, 40).

In conclusion, the nature of the antigen at a given site directs the IgA subclass distribution of the immune response to these antigens, with polysaccharide antigens shifting the distribution toward IgA2 and proteins toward IgA1. The locally activated lymphocytes express certain sets of HR and CCR which enable these cells to home back to mucosal sites after their circulation through lymphatics and blood. Consistent with the high intestinal polysaccharide and LPS loads, the concentration of IgA2 is higher in the intestine and the proportion of gut HR-expressing cells more frequent among IgA2 plasmablasts than among IgA1 plasmablasts. Hence, together with the nature of the antigen, the site-specific homing of IgA1 and IgA2 plasmablasts is a key factor responsible for the differential distribution of IgA subclasses in the body fluids.

FIG. 4. Arithmetic means (± standard deviations) of the percentages of antigen-specific IgA1- and IgA2-secreting cells expressing the indicated HR in healthy volunteers after oral vaccination with live attenuated Salmonella serovar Typhi Ty21a (A) or parenteral vaccination with an unconjugated pneumococcal polysaccharide vaccine (B). The numbers of volunteers from whom the data were pooled are given under the bars. There were no statistically significant differences between IgA1 and IgA2 ASC in the paired Student’s t test.
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