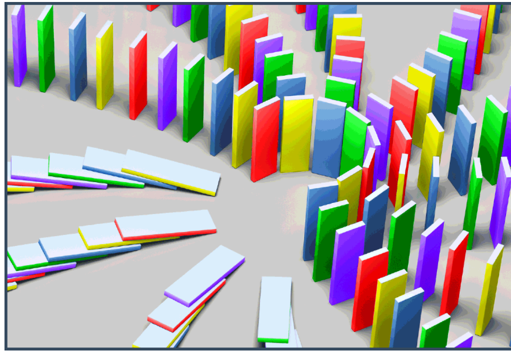

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A Common Mucosal Chemokine (Mucosae-Associated Epithelial Chemokine/CCL28) Selectively Attracts IgA Plasmablasts[1]

Nicole H. Lazarus, Eric J. Kunkel, Brent Johnston, Eric Wilson, Kenneth R. Youngman, and Eugene C. Butcher[2]

IgA immunoblasts can seed both intestinal and nonintestinal mucosal sites following localized mucosal immunization, an observation that has led to the concept of a common mucosal immune system. In this study, we demonstrate that the mucosae-associated epithelial chemokine, MEC (CCL28), which is expressed by epithelia in diverse mucosal tissues, is selectively chemotactic for IgA Ab-secreting cells (ASC): MEC attracts IgA- but not IgG- or IgM-producing ASC from both intestinal and nonintestinal lymphoid and effector tissues, including the intestines, lungs, and lymph nodes draining the bronchopulmonary tree and oral cavity. In contrast, the small intestinal chemokine, TECK (CCL25), attracts an overlapping subpopulation of IgA ASC concentrated in the small intestines and its draining lymphoid tissues. Surprisingly, T cells from mucosal sites fail to respond to MEC. These findings suggest a broad and unifying role for MEC in the physiology of the mucosal IgA immune system. ***The Journal of Immunology,* 2003, 170: 3799–3805.**

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The mammalian immune system is poised to block pathogen entry at epithelial surfaces through a combination of humoral and cell-mediated defenses. Epithelial tissues contain various populations of resident T- and B-lymphocytes, dendritic cells, macrophages, and granulocytes that survey the microenvironment for pathogens. At mucosal epithelial surfaces, the luminal transport of IgA secreted from resident plasma cells is a major mechanism of pathogen neutralization and host protection (1, 2). Although the upper respiratory tract, oral cavity, gastrointestinal tract, and genital tract are in direct contact with the environment, mucosal surfaces at these sites serve to protect from infection by similar environmental pathogens, making the ability to transfer IgA-dependent immunity between such sites critical to host defense.

Several lines of evidence support the existence of such a common mucosal immune system for Ab-secreting B cells. Peyer's patch (PP)[3] lymphocytes can selectively reconstitute the IgA Ab-secreting cell (ASC) compartment of the intestinal and bronchial lamina propria in irradiated rabbits (3, 4). In addition, transferred intestinal IgA immunoblasts from the mesenteric lymph node (MLN) can seed not only the small intestine, but also the respiratory tract, genital tract, and mammary gland (5). This ability of IgA immunoblasts from the intestinal associated lymphoid tissues to migrate to and populate nonintestinal mucosal sites correlates well with the findings that oral Ag administration leads to Ag-specific IgA in both intestinal (e.g., stool) and nonintestinal (e.g., saliva, milk, vaginal secretions) sites (6).

Nevertheless, the mechanisms controlling the localization of IgA immunoblasts in such diverse mucosal tissues are not well understood. We have recently demonstrated that IgA ASC from the spleen, intestinal lymphoid tissues, and the small intestine itself are attracted by thymus-expressed chemokine (TECK/CCL25) (7), a CCR9 chemokine ligand expressed by small intestinal epithelial cells (8, 9), and a chemoattractant for circulating and resident gut-homing T cells (8, 9). TECK and CCR9 appear to contribute to the segregation of the small intestinal T cell compartment because very few $CCR9^+$ memory T cells are found in nonlymphoid tissues outside of the small intestine (9), and TECK may play a similar role for IgA ASC (7). However, IgA ASC populate diverse mucosal tissues, and a previous study has demonstrated that mucosal IgA-secreting B cells from intestinal lymphoid tissues can chemotax in response to a factor present in mouse colostrum (10). Because TECK itself is not highly expressed by epithelial cells in mucosal sites outside of the small intestine (9) (e.g., mammary gland, salivary gland, colon, lung, and trachea), we hypothesized that another chemotactic factor or factors for IgA ASC may be present in these mucosal epithelial tissues.

We now show that the recently described mucosae-associated epithelial chemokine (MEC/CCL28) (11, 12), a CCR10 ligand expressed by epithelial cells in diverse mucosal sites, is a potent chemoattractant for IgA ASC. MEC, which is expressed in the salivary gland, colon, and by bronchial and mammary gland

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[3] Abbreviations used in this paper: PP, Peyer's patch; ASC, Ab-secreting cell; BCS, bovine calf serum; CLA, cutaneous lymphocyte Ag; CTACK, keratinocyte-expressed cutaneous T cell attractant chemokine; int, intermediate; LPL, lamina propria lymphocyte; MEC, mucosae-associated epithelial chemokine; MLN, mesenteric lymph node; SDF-1α, human stromal cell-derived factor-1α; SGLN, lymph nodes draining submandibular gland and oral and nasal cavities; TBLN, tracheo-bronchial lymph nodes draining lungs and trachea; TECK, thymus-expressed chemokine; int, intermediate.

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epithelium, efficiently attracts IgA, but not IgG or IgM ASC. In contrast to TECK, whose restricted expression in the small intestine correlates with an ability to attract IgA ASC preferentially from the intestines and its draining lymphoid tissues, MEC attracts IgA ASC from diverse mucosal lymphoid organs and from both intestinal and extraintestinal (e.g., pulmonary) mucosal tissues. The ability to migrate to MEC thus appears to be a defining and unifying feature of IgA ASC, and is likely to contribute to the dissemination of the secretory IgA Ab response throughout the mucosal immune system.

Materials and Methods

Animals

Female C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice 6–8 wk old were used for most experiments, except for analysis of IgA ASC chemotaxis from the salivary gland lymph nodes draining the submandibular gland and nasal and oral cavities (SGLN) and tracheobronchial lymph nodes (TBLN), in which 4- to 6-mo-old mice were used because of the presence of more IgA ASC. Chemotaxis of spleen IgA ASC was not significantly different between the two age groups.

Lung hypersensitivity induction

A generalized lung hypersensitivity response was induced as described (13). Briefly, female C57BL/6 mice were primed by an i.p. injection of 1×10^8 SRBCs (Colorado Serum, Denver, CO), and 2 wk later, the mice were challenged intratracheally with 5×10^8 SRBCs. Three days later, mice were sacrificed and bronchial alveolar lavage was performed with 1 ml saline to isolate cells from the bronchials and alveolae. After lavage, lung lymphocytes were isolated, as described below, and both cell populations were analyzed for chemokine responsiveness by ELISPOT and FACS.

Cell isolation

Mice were sacrificed by CO_2 inhalation. Lymphocytes from lymphoid tissues (spleen, PP, MLN, SGLN, and TBLN) were isolated by mechanical dispersion through a wire mesh, followed by hypotonic RBC lysis. Before the isolation of whole lung lymphocytes, all associated lymph nodes were carefully removed. The lung tissue was minced and subsequently digested in RPMI 1640 supplemented with 300 U/ml collagenase type VIII (Sigma-Aldrich, St. Louis, MO) for 30 min at 37°C, followed by hypotonic RBC lysis. Lamina propria lymphocytes (LPL) were isolated from the small intestine, as described (14), with modifications. Briefly, five to six intestines were cleared of PP, cut open longitudinally, cut into short 5-mm segments, and washed four to five times in Ca^{2+}/Mg^{2+}-free HBSS supplemented with 1 mM EDTA, 15 mM HEPES, and fungicide/antibiotic, to remove epithelial cells and intraepithelial lymphocytes. Intestines were then washed in RPMI 1640 with 10% bovine calf serum (BCS), 15 mM HEPES, and fungicide/antibiotic to neutralize the EDTA. LPL were isolated by shaking the intestinal pieces in RPMI 1640 supplemented with 300 U/ml collagenase type VIII (Sigma-Aldrich), 10% BCS, and fungicide/antibiotic for three 40-min sessions at 37°C. All lymphocytes were allowed to recover in RPMI 1640 with 10% BCS in a 5% CO_2 incubator for 1–2 h before chemotaxis analyses.

Chemotaxis assays

Chemotactic migration assays were performed, as previously described (15). Briefly, between 1×10^5 and 2×10^6 lymphocytes from different tissues (depending on the frequency of IgA ASC) were placed in the upper chamber of 5-μm Transwell inserts (Corning Costar, Cambridge, MA), and inserts were placed in wells with medium alone (basal) or medium containing 100 nM human stromal cell-derived factor-1α (SDF-1α/CXCL16), 250 nM human MEC or murine MEC, or 300 nM murine TECK. All chemokines were obtained from R&D Systems (Minneapolis, MN) and used at the optimal concentration, as determined by titration on PP, MLN, and splenic IgA ASC. IgA ASC responded to human MEC, murine MEC, and TECK with similar dose responses (data not shown). After 2 h, inserts were removed, and the responding populations in the bottom well were quantified by FACS or ELISPOT analysis. In serial chemotaxis assays, cells were initially migrated to one chemokine in multiple wells, and the migrated cells from three chemotaxis wells were pooled and allowed to resensitize for 1 h at tissue culture conditions, and then migrated a second time to the same or a different chemokine.

ELISPOT assays

IgA, IgG, or IgM ASC were identified by conventional ELISPOT analysis using nitrocellulose 96-well plates (Multiscreen 96-well filtration plate; Millipore, Bedford, MA) that were coated overnight with goat anti-mouse IgA-, IgG-, or IgM-specific polyclonal Abs (Kirkegaard & Perry, Gaithersburg, MD). Cells were incubated in the wells overnight and removed, and the captured Ig was detected with HRP-conjugated goat anti-mouse IgA, IgG, or IgM (Kirkegaard & Perry), and visualized by development with 3-amino-9-ethylcarbazole for 15–20 min to yield a reddish spot where an ASC had sat and secreted Ab. The percentage of migration was calculated by comparing the number of ASC in the input population (plated and enumerated in a similar manner) with the number of spots present in the migrated populations.

Flow cytometry

Migrated lymphocytes were enumerated using a bead-counting method, as described (15). The following rat anti-mouse Ab conjugates were used in the various staining protocols: B220 APC (clone RA3-6B2); TCRβ FITC (clone H57-597); CD4 PE (clone RM4-5); CD8α PerCP (clone 53-6.7); CD138 PE (clone 281-2); CD19 PE (clone 1D3); IgA FITC (clone C10-3); IgA biotin (clone C10-1); Thy-1.2 PE or biotin (clone 53-2.1); IgD biotin (clone 11-26); NK1.1 PE or biotin (clone PK136); CD11c PE or biotin (clone HL3); F4/80 PE (clone CI:A3-1); IgM FITC (clone II/41); and IgG1 FITC (clone A85-1) were all from BD PharMingen (San Diego, CA). IgD PE (clone 11-26) was from Southern Biotechnology Associates (Birmingham, AL), and donkey anti-human IgG PE was from Jackson ImmunoResearch (West Grove, PA). Biotin-conjugated mAbs were detected with streptavidin PerCP (BD PharMingen). Four-color flow cytometry was done on a FACSCalibur (BD Biosciences, San Jose, CA) using CellQuest software, version 3.1 (BD Biosciences).

MEC Fc-chimera generation and binding assay

The coding region of murine MEC was isolated by PCR using a 5′ primer containing an *Nhe*I site and a 3′ primer with a *Bam*HI site. This fragment was inserted into a human IgG vector (gift from B. Seed, Harvard University, Boston, MA) to generate a chimeric MEC-Fc construct. The MEC-Fc chimera was transiently transfected into CHO-P cells using Lipofectamine, as per the manufacturer's recommended protocol (Invitrogen, Carlsbad, CA), and after 4-day culture, MEC-Fc-containing supernatants were collected. Splenocytes were incubated with the MEC-Fc supernatant for 20 min at 4°C and washed. The cells were subsequently stained with the following Ab mixture: donkey anti-human IgG PE, Ig lineage-specific Abs (rat anti-mouse IgA, IgG1, or IgM), B220 APC, and biotin-conjugated Thy-1.2, IgD, NK1.1, and CD11c (dump gate). To confirm MEC-Fc chimera-binding specificity, 5 μg polyclonal goat anti-mouse MEC/CCL28 (R&D Systems) was used to inhibit MEC-Fc binding.

Intracellular staining for IgA

LPL were isolated, as described above, and further purified by density-gradient centrifugation through 44% Percoll (Amersham Biosciences, Piscataway, NJ). Cells were incubated with F4/80 PE, Thy-1.2 PE, IgD PE, NK1.1 PE, CD11c PE (dump gate), IgA FITC, and B220 APC. Cells were then fixed and permeabilized using Cytofix/Cytoperm (BD PharMingen) and subsequently incubated with anti-IgA biotin in Perm/Wash buffer (BD PharMingen), followed by incubation with streptavidin PerCP. Absence of surface IgA staining by biotinylated anti-IgA was verified by incubating unpermeablized, paraformaldehyde-fixed, anti-IgA FITC-stained cells with anti-IgA biotin, followed by streptavidin PerCP. PerCP fluorescence was equivalent to levels seen using isotype control biotin mAb.

Statistical methods

Student's t test was used to analyze the results, and $p < 0.05$ was considered significant.

Results and Discussion

The strong sequence homology between MEC and the intestinal chemokine TECK, an attractant for IgA ASC (7), together with the expression of MEC in epithelial tissues (e.g., colon, mammary gland, salivary gland, lung, and trachea) in which IgA ASC are present (11), led us to hypothesize that MEC may also be a chemoattractant for IgA ASC. Therefore, we tested the ability of MEC to attract IgA ASC from a variety of murine lymphoid tissues by chemotaxing lymphocytes in a Transwell assay and enumerating

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migrated cells with a functional Ig isotype-specific ELISPOT assay (Fig. 1*A*). IgA ASC from the spleen (a central filtering lymphoid organ through which most circulating lymphocyte subsets pass) responded to TECK, as shown previously (7), but also robustly to MEC. In addition, splenic IgA ASC responsiveness to keratinocyte-expressed cutaneous T cell attractant chemokine (CTACK/CCL27), the second known CCR10 ligand, was tested (19 and 21% migration in the two experiments performed) and found to be comparable to the MEC response (18–24%). Although we previously demonstrated a chemotactic response to TECK by IgA ASC from small intestinal PP and a slightly lower migration of MLN (which drains the small and large intestines) (7), we found that these intestinal IgA ASC also migrate to MEC as well as or better than to TECK (Fig. 1*A*). Moreover, IgA ASC from nonintestinal mucosae-draining lymph nodes (the SGLN that drains the submandibular salivary glands and oral and nasal cavities, and the TBLN that drains the trachea and lungs) migrated well to MEC, but poorly, if at all, to TECK (Fig. 1*A*), a finding that correlates with the expression of ample MEC, but little or no TECK in salivary glands and bronchopulmonary tissues (9, 11). This differential MEC responsiveness of IgA ASC from nonintestinal lymph nodes is emphasized by the calculated ratio of the efficiency of migration to MEC vs TECK. A ratio close to 1 indicates that the migration of resident IgA ASC to MEC and TECK is similar (e.g., 1.5 in the PP and 2 in the MLN), while a ratio above 1 indicates that MEC is much more efficient (e.g., 14 in the SGLN and 15 in the TBLN). Thus, whereas the response to TECK is primarily limited to intestinal associated lymphoid tissues (the PP and MLN), robust responsiveness

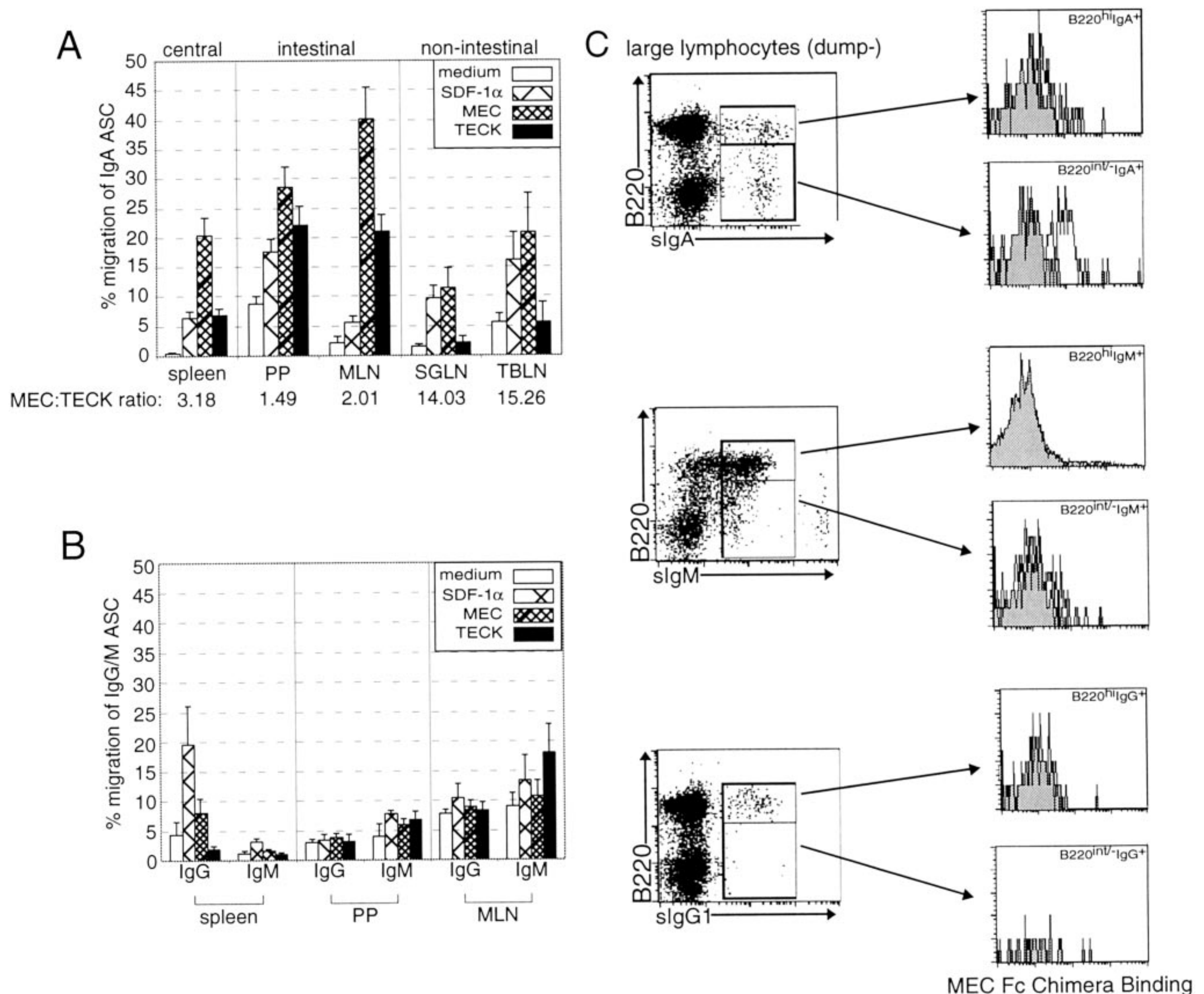

FIGURE 1. MEC/CCL28 attracts lymphoid tissue IgA ASC. Lymphocytes from the spleen (a central multisystem lymphoid tissue), PP (small intestine-associated lymphoid tissue), MLN (draining the large and small intestines), SGLN (draining the submandibular glands and oral and nasal cavities), and TBLN (draining the trachea and bronchial tree) were migrated to medium, 100 nM human SDF-1α, 250 nM human MEC, or 400 nM human TECK, and enumerated with an Ig isotype-specific ELISPOT. The number of IgA ASC per 1 million lymphocytes obtained from the tissues is as follows: spleen 106 ± 11, PP 196 ± 30, MLN 95 ± 7, SGLN 153 ± 59, and TBLN 78 ± 32. IgA ASC from all lymphoid tissues migrated to both MEC and TECK (*A*), although the highest TECK response was associated with intestinal lymphoid tissues (ratio in *A*). Splenic or MLN IgG and IgM ASC did not migrate well to either MEC or TECK (*B*). MEC-Fc fusion protein was used to stain splenocytes to determine MEC-Fc-binding patterns. MEC-Fc fusion protein bound $B220^{int/-}IgA^+$, but not IgG^+ or IgM^+ cells (*C*). The specificity of MEC-Fc binding (open histograms) is shown by the addition of an anti-MEC Ab that inhibits MEC-Fc/CCR10 binding (shaded histograms). Data shown as the mean ± SEM of at least three experiments from different days on lymphocytes pooled from several mice for each lymphoid tissue. In *A*, migration of all subsets to each chemokine tested was significantly above background ($p < 0.05$). In *B*, only IgG ASC migration to SDF-1α was significantly above migration to medium.

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to MEC is a common feature of IgA ASC from all lymphoid tissues tested (Fig. 1*A*). In these experiments, IgA ASC from all lymphoid tissues also migrated to SDF-1α, a chemokine that attracts most lymphocytes and that, in the context of ASC migration, has been implicated in the recruitment of ASC to the bone marrow (16).

In contrast to the robust response of IgA-producing cells, IgG and IgM ASC from spleen, PP, and MLN do not respond well to MEC (or TECK), although splenic IgG ASC responded well to SDF-1α, consistent with previous findings (16). Thus, MEC responsiveness is largely, if not exclusively, limited to IgA ASC (Fig. 1*B*). Consistent with these functional responses, we found that most splenic IgA (but not IgG or IgM) ASC bound a MEC-Fc chimeric protein (Fig. 1*C*).

Previous studies in the human have shown that MEC can signal through both CCR10 and CCR3; indeed, human rMEC attracts human blood eosinophils in a CCR3-dependent fashion (11). However, we have not observed chemotaxis of mouse eosinophils to MEC (data not shown). Moreover, IgA ASC do not migrate to eotaxin (data not shown), a specific CCR3 ligand (17). Recent studies in the human show that almost all IgA ASC present in the salivary gland, small intestine, and large intestine stain with an Ab to CCR10. In contrast, IgG^+ cells present in these same tissues were virtually negative for CCR10 expression.[4] We conclude that CCR10 is preferentially and selectively expressed by IgA ASC.

The observation that IgA ASC from intestine-associated lymphoid tissues respond to both MEC and TECK suggests that either the same population of IgA ASC can respond to both chemokines, or that two distinct or partially overlapping populations of IgA ASC are present, one that responds to MEC and thus may be targeted to mucosal sites outside the small intestine, and one that responds to TECK. To address this issue, we conducted serial chemotaxis experiments. Lymphocytes from the MLN were first migrated to MEC or TECK (Fig. 2*A*); the migrated populations were harvested and allowed to rest for 1 h at tissue culture conditions to restore chemokine responsiveness, and then migrated again to medium, SDF-1α, MEC, or TECK, and enumerated by ELISPOT assay (Fig. 2*B*). Lymphocytes from the MLN were chosen for serial chemotaxis because of their robust responsiveness to both chemokines (Fig. 1*A*) and the previous data showing that MLN IgA immunocytes were able to repopulate both the intestines and nonintestinal mucosal tissues (5). As shown in Fig. 2*A*, IgA ASC that migrate to MEC (or TECK) retain the ability to migrate to the same chemokine in secondary migration assays (Fig. 2*B*). The ability of a fraction of cells first migrated to MEC to subsequently respond to TECK, and vice versa, shows that a population of IgA ASC in the MLN exists that can respond to both chemokines. The serial migration data also suggest that populations of IgA ASC exist that may be responsive only to MEC, because if all the IgA ASC were dually responsive, we would expect no difference in the migratory response of MEC-enriched or TECK-enriched IgA ASC to TECK in the second migration. Instead, cells migrated to TECK show dramatically enhanced secondary migration to TECK, suggesting enrichment of a subset. Conversely, whether migrated to TECK or MEC, or assessed without prior migration, IgA ASC show a similar efficiency of migration to MEC, suggesting a fairly uniform responsiveness. Consistent with this interpretation, we find that almost all IgA ASC migrated to TECK stain with the MEC-Fc chimera (data not shown). In combination with the selective responsiveness of TBLN and SGLN IgA ASC to MEC, these serial chemotaxis findings suggest that responsiveness to

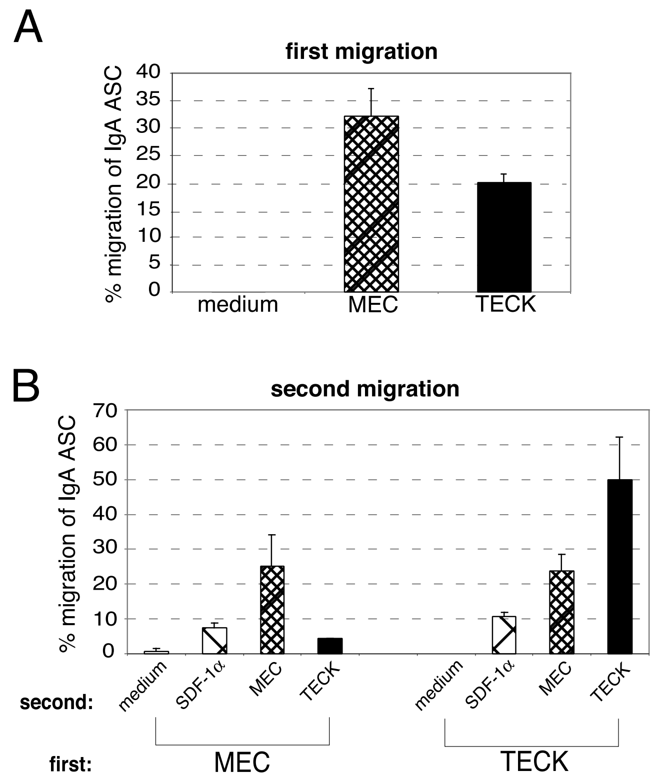

FIGURE 2. Overlapping populations of IgA ASC migrate to TECK and MEC. Lymphocytes from the MLN were initially migrated to medium, MEC, and TECK (*A*), rested for 2 h at 37°C to restore chemokine responsiveness, then migrated a second time to medium, SDF-1α, MEC, and TECK, and the responding IgA ASC enumerated by ELISPOT (*B*). IgA ASC initially migrated to MEC responded well to MEC in a second migration, but less well to TECK. IgA ASC initially migrated to TECK migrated to both TECK and MEC the second time. The ability of the TECK-responsive IgA ASC to migrate well to both MEC and TECK suggests that many or most TECK-responsive IgA ASC also respond to MEC. Data shown as mean ± SEM of migration in three replicate wells and are representative of two independent experiments performed from MLN pooled from 20 mice. Migration to each chemokine was significantly above background ($p < 0.05$).

MEC and TECK can be independently regulated during the differentiation of IgA ASC, that is, IgA ASC in gut-associated lymphoid tissues frequently express receptors for both the small intestinal chemokine TECK and the broadly expressed mucosal epithelial chemokine MEC. This explanation is supported by emerging data in the human showing that CCR10 (the receptor for MEC) is expressed on almost all IgA plasmablasts and plasma cells resident in many mucosal tissues and lymphoid organs, including the tonsil, salivary gland, stomach, small intestine, appendix, and large intestine, while expression of CCR9 (the receptor for TECK) is predominantly restricted to a subset of IgA plasma cells in the small intestine.[4]

We next determined whether IgA ASC resident in extralymphoid mucosal effector sites were also responsive to MEC. IgA ASC are abundant in the lamina propria of the small intestine, allowing evaluation of chemotaxis (and the phenotype of the migrated cells) by FACS. Small intestinal LPL were migrated to medium, SDF-1α, MEC, or TECK, and the migration was enumerated by flow cytometry. In the small intestine, MEC was chemotactic for a population of $sIgA^+B220^{int}$ large B cells (Fig. 3*A*) that were also $CD138^+$ (data not shown). This phenotype is similar to that of ASC that respond to TECK (7). The more abundant population of $sIgA^+B220^-$ B cells did not respond well to

[4] E. J. Kunkel, C. H. Kim, M. A. Vierra, D. Soler, E. P. Bowman, and E. C. Butcher. CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA antibody secreting cells. *Submitted for publication*.

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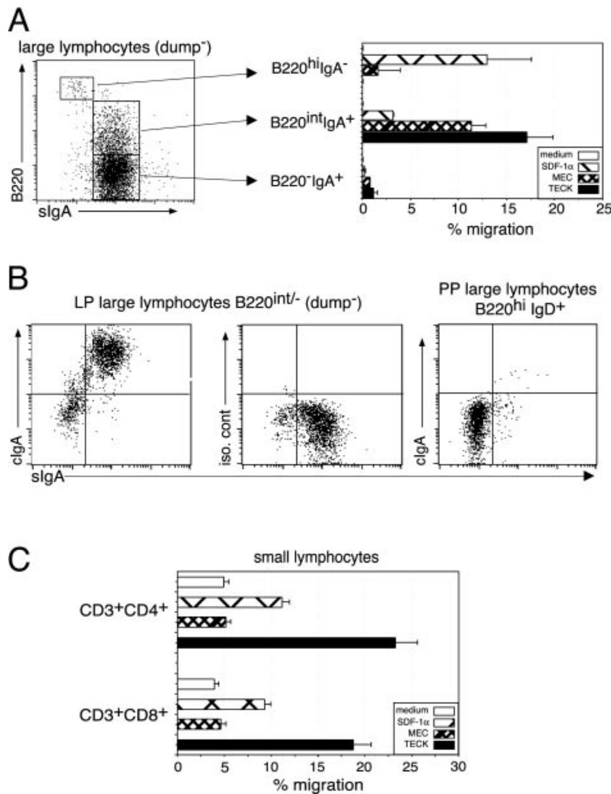

FIGURE 3. Small intestinal sIgA$^+$B220^{int} plasmablasts (but not mature plasma cells or T cells) migrate efficiently to MEC. *A*, Small intestinal LPL were isolated and migrated to medium, SDF-1α, MEC, or TECK, and migrated subsets were enumerated by FACS analysis. Large B cells were enriched by gating on Thy-1.2$^-$IgD$^-$CD11c$^-$NK1.1$^-$ cells (dump$^-$) and then analyzed based on B220 vs sIgA staining. Only sIgA$^+$B220^{int} large B cells (plasmablast phenotype) migrated to MEC (and to TECK) significantly above background ($p < 0.05$). The resident plasma cells (sIgA$^+$B220$^-$ B cells) did not migrate well to any chemokine. Large sIgA$^-$B220^{high} lymphocytes responded only to SDF-1α. *B*, All surface IgA$^+$B220$^{int/-}$ small intestinal LPL contain high levels of intracellular IgA, and thus represent ASC. *C*, Small intestinal T cells were defined as CD3$^+$CD4$^+$ or CD3$^+$CD8$^+$ small lymphocytes. No T cells from the small intestine migrated to MEC, although the same cells migrated to TECK and SDF-1α. Data shown as mean ± SD of migration in three replicate wells and are a representative experiment of three total.

any chemokine (Fig. 3*A*). Previous studies indicate that the B220^{int} (migrating) subset are plasmablasts, while the B220$^-$ (nonresponsive) population comprises more terminally differentiated mature tissue plasma cells (7, 18). The chemokine responsiveness of sIgA$^-$B220^{high} cells is shown for contrast: these presumably naive or memory B cells respond to SDF-1α, but not to the epithelial chemokines MEC or TECK. Importantly, all of the sIgA$^+$B220$^{int/-}$ LPL contain high levels of intracellular IgA (Fig. 3*B*), confirming that they are ASC. In addition, IgA ASC from the lamina propria of the large intestine were analyzed for chemotaxis. Similar to the migratory response of IgA ASC from the small intestine LPL, sIgA$^+$B220^{int} large B cells migrated, while sIgA$^+$B220$^-$ B cells did not respond (data not shown). Together, these observations support the conclusion that intestinal IgA ASC can respond to both MEC and TECK, and that the predominantly responsive cells are sIgA$^+$B220^{int} plasmablasts, which may represent ASC more recently recruited from circulating precursors.

T cells from the small intestine lamina propria were also examined for MEC responsiveness. As shown in Fig. 3*C*, neither CD3$^+$CD4$^+$ nor CD3$^+$CD8$^+$ T cells from the small intestine respond to MEC, even though they migrate robustly to TECK and SDF-1α. We conclude that MEC is a remarkably selective attractant for IgA ASC.

We next assessed chemotaxis of IgA ASC from a nonintestinal mucosal tissue, the lung. Lymphocytes were isolated from the lung and migrated to medium, SDF-1α, MEC, or TECK, and the phenotype of the responding cells was determined by flow cytometry (Fig. 4*A*). Similar to the results with small intestinal cells, the migratory large B cells were sIgA$^+$B220^{int} (although evaluation of the chemotaxis of resident mature phenotype sIgA$^+$B220$^-$ plasma cells in the lung was not possible, because of their low freqency). However, in contrast to intestinal IgA plasmablasts, these pulmonary sIgA$^+$B220^{int} plasmablasts responded strongly to MEC, but only poorly to TECK. ELISPOT analyses confirmed that IgA ASC isolated from lung tissue displayed a strong response to MEC, but only a small response to TECK (Fig. 4*B*).

To assess potential alterations in ASC responses in pulmonary inflammation, a model of general lung hypersensitivity was used. Briefly, 2 wk after i.p. priming, mice were challenged intratracheally with SRBCs, and bronchopulmonary tissue lymphocytes were isolated for analysis. As seen in Fig. 4*B*, IgA ASC from inflamed lung responded robustly to MEC.

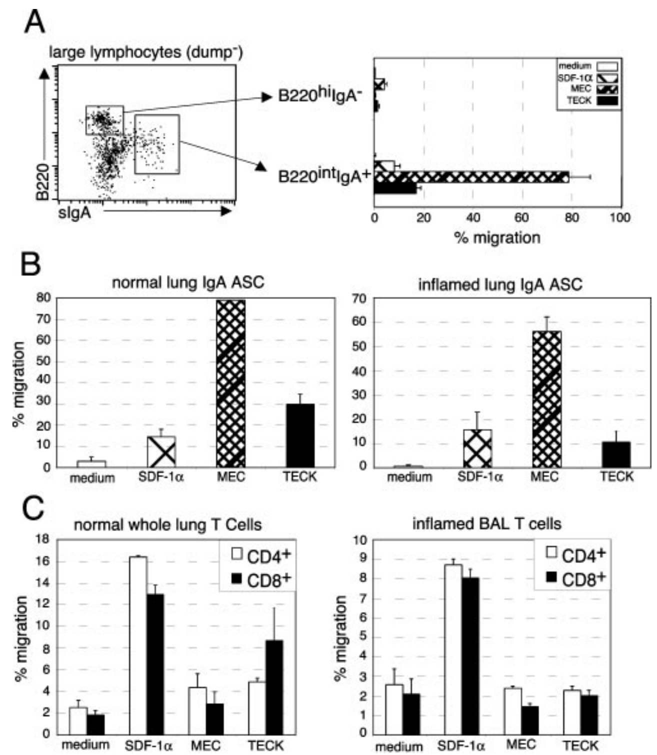

FIGURE 4. MEC attracts sIgA$^+$B220^{int} plasmablasts from normal and inflamed lung. *A*, Lymphocytes from normal lung were isolated and migrated to medium, SDF-1α, MEC, or TECK, and the migrated populations were analyzed by FACS. Large B cells were enriched by gating on Thy-1.2$^-$IgD$^-$CD11c$^-$NK1.1$^-$ cells (dump$^-$) and then gating on B220 vs sIgA. sIgA$^+$B220^{int} large B cells (plasmablasts) migrated well to MEC (and poorly to TECK). Large sIgA$^-$B220^{high} cells responded only to SDF-1α. *B*, By ELISPOT, functional IgA ASC from normal and SRBC-inflamed lung responded robustly to MEC and, to a lesser degree, to TECK. *C*, Resident T cells from normal lung failed to respond to MEC, and T cells recently recruited to the SRBC-inflamed lung, isolated from bronchial-alveolar lavage fluid, also failed to respond to MEC, although T cells from both normal and inflamed lung responded to SDF-1α. Data shown as the mean ± SEM of three independent experiments performed on lung lymphocytes pooled from 10–15 mice ($p < 0.05$ as compared with migration to medium).

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Finally, we also examined the chemotactic responses of both resident normal lung T cells and recently recruited T cells residing in the bronchial-alveolar compartment of inflamed lung. T cells from both normal lung and elicited to bronchial-alveolar lavage were unresponsive to MEC, although they responded well to SDF-1α (Fig. 4*C*). Thus, IgA ASC from both normal and inflamed bronchopulmonary tissues migrate efficiently to MEC, while most T cells fail to respond.

Taken together, our findings establish MEC as a potent and selective chemoattractant for IgA ASC. Although, as previously shown, IgA ASC from intestinal lymphoid and effector sites respond to TECK (7), MEC elicits a strong migratory response of IgA ASC from both intestinal and extraintestinal mucosal lymphoid and effector sites, including bronchopulmonary tissues and lymph nodes draining the salivary glands. Thus, the physiologic migratory response to MEC provides a potential mechanism for widespread mucosal dissemination of IgA ASC.

It is worthwhile considering our results in the context of previous studies that have examined the homing potential of IgA immunoblasts or their immediate precursors from a variety of lymphoid organs. Cells isolated from gut-associated lymphoid tissues (e.g., MLN and PP) have been shown to repopulate both intestinal and nonintestinal sites with IgA-secreting cells (3, 4, 19). This ability of cells from the gut to migrate to and seed multiple tissues with IgA ASC may in part reflect the dual responsiveness, shown in this study, of IgA plasmablasts to MEC and TECK. Conversely, TBLN immunoblasts efficiently repopulate the lungs and, to a lesser extent, the salivary gland (5, 19), but not the small intestine. In addition, IgA ASC from MLN have been shown to be responsive to a chemotactic factor found in the colostrum of milk (10), which may relate to the high level of MEC expression in the mammary gland (11). Together, the correlation of these immunoblast homing studies (3, 4, 5, 6, 19) with our functional analyses of IgA ASC chemotactic responses is consistent with the potential importance of MEC and TECK in the distribution and targeting of IgA-producing cells. The general ability of IgA ASC to respond to MEC may thus help explain the transfer of IgA-dependent immunity to specific Ags between the intestines and distant sites such as the oral cavity and mammary gland, or between the nasal cavity and genital tract (5, 6). For example, localized nasal or oral vaccination is known to lead to IgA-dependent pathogen protection at multiple mucosal sites (1, 20, 21).

Of course, any model of physiologic ASC dissemination and localization must also include the role of tissue-selective adhesion pathways involved in lymphocyte recruitment, and of local Ag involved in Ag-specific ASC retention and proliferation (22). These mechanisms are likely to act in conjunction with epithelial chemokine activities in the differential repopulation of IgA ASC responses in vivo. Further exploration of the molecular mechanisms behind IgA-secreting plasmablasts trafficking between distant mucosal sites will be critical to an understanding of the dissemination of secretory IgA immunity following localized vaccination or infection.

Although T cells from mucosal tissues were unresponsive to MEC, MEC is chemotactic for circulating memory T cells expressing cutaneous lymphcoyte Ag (CLA) defined as skin-homing memory T cells in human blood: these cells respond to the homologous CCR10 ligand CTACK/CCL27, expressed by skin keratinocytes (23). Indeed, recent studies confirm that CTACK is an important mediator of T cell recruitment to inflamed skin in delayed-type hypersensitivity models (24). The specialized role for CTACK/CCR10 in cutaneous T cell trafficking, and of MEC/CCR10 in mucosal IgA responses, is understandable within the context of the multistep process of lymphocyte homing (25). Circulating CLA^+ skin memory cells lack the intestinal homing receptor $\alpha_4\beta_7$ (26, 27), and thus cannot interact with the intestinal venules that express the $\alpha_4\beta_7$ ligand mucosal addressin cell adhesion molecule-1 (28, 29); thus, skin-homing CLA^+ memory T cells would not have the opportunity to respond to MEC in intestinal sites. CTACK expression by keratinocytes has the potential to participate in the recruitment of $CCR10^+$ IgA ASC to the skin (as secretory IgA is in fact found in sweat (30)), but again, the engagement of circulating IgA plasmablast CCR10 would require and be regulated by appropriate adhesion homing receptor coexpression for interacting with cutaneous vessels (e.g., CLA, $\alpha_4\beta_1$, and LFA-1).

In conclusion, our results suggest a unique and unifying role for the mucosal epithelial chemokine in the biology of mucosal IgA Ab secretion. The ability of MEC to attract migratory IgA ASC from all sites examined, in conjunction with its expression by epithelial cells in diverse mucosal tissues, provides a potential explanation for the widespread dissemination of secretory IgA responses following local mucosal antigenic stimulation, the defining feature of the common mucosal immune system.

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