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The differential expression of chemokines and chemokine receptors, by tissues and leukocytes, respectively, contributes to the specific accumulation of leukocyte subsets to different tissues. CCR10/CCL28 interactions are thought to contribute to the accumulation of IgA Ab-secreting cells (ASC) to mucosal surfaces, such as the gastrointestinal tract and the lactating mammary gland. Although the role of CCL28 in lymphocyte homing is well established, direct in vivo evidence for CCR10 involvement in this process has not been previously shown. In this study, we describe the generation of a CCR10-deficient mouse model. Using this model, we demonstrate that CCR10 is critical for efficient localization and accumulation of IgA ASC to the lactating mammary gland. Surprisingly, IgA ASC accumulation to the gastrointestinal tract is minimally impacted in CCR10-deficient mice. These results provide the first direct evidence of CCR10 involvement in lymphocyte homing and accumulation in vivo, and demonstrate that reliance on CCR10-mediated recruitment of IgA ASC varies dramatically within mucosal tissues. The Journal of Immunology, 2008, 181: 6309–6315.

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Mucosal surfaces of the body are continually exposed to a wide variety of pathogens and toxins; thus, immune protection of these surfaces is of utmost importance to host survival. Protection of mucosal surfaces is dependent on efficient homing and accumulation of effector αβ and γδ T cells, as well as Ab-secreting cells (ASC). IgA is the most common Ab isotype involved in the protection of human and mouse mucosal surfaces. In the gastrointestinal tract, IgA ASC accumulate in the lamina propria, where they secrete Abs that are transcytosed across the intestinal epithelium into the lumen. Secreted IgA Abs bind and neutralize pathogens and toxins within mucosal epithelial cells and within the gut lumen, thus affording mucosal immune protection to the host (1–3).

Infants are born with naive immune systems, and are thus at increased risk of infection until their immune system generates memory/effector lymphocytes. Passive immune protection of the newborn gastrointestinal tract is dependent on an active process of IgA ASC accumulation in the lactating mammary gland of the mother (4–7). Through this process, IgA Abs specific to pathogens in the mother’s environment are secreted into the milk and passively transferred to the gut of the suckling neonate. This process provides the infant with Ag-specific protection against gastrointestinal pathogens previously encountered by the mother (8).

The efficient migration and accumulation of lymphocytes are highly dependent on tissue-expressed chemokines and leukocyte-expressed chemokine receptors. The chemokine receptor CCR10 is thought to help direct subsets of lymphocytes to mucosal and cutaneous tissues via interactions with the chemokines CCL28 and CCL27, respectively. However, this hypothesis is based not on studies of CCR10 itself, but rather on studies using neutralizing Abs against CCL27 and CCL28 (7, 9–13). Complicating the study of CCR10/ligand interactions are data suggesting the interaction of CCL28 with CCR3 (14, 15). CCL28 and CCL27 could also bind additional chemokine receptors through interactions that have not yet been characterized.

IgA ASC have been hypothesized to traffic to multiple mucosal sites via a common mucosal immune system (16). Such a system would afford protection to a broad spectrum of mucosal tissues after immunization to a single mucosal site. Evidence for the common mucosal immune system is partly based on data showing that IgA immunoblasts collected from mesenteric lymph nodes efficiently seed the gastrointestinal tract, mammary gland, respiratory tract, and genital tract (16). CCR10 interactions are of particular interest to mucosal immunity in that CCL28 is expressed in a broad range of mucosal tissues, and has been hypothesized to act as a unifying factor in the common mucosal immune system (14, 17).

In an effort to define the role of CCR10 in lymphocyte homing to mucosal tissues, we selected a direct approach: targeted depletion of CCR10 expression in mice. In this study, we describe the engineering of mice genetically deficient in functional CCR10. The role of CCR10-mediated homing was then tested using a lactating mouse mammary gland model. This model is ideal in that IgA ASC isolated from the mammary gland have been shown to lack...
mRNA expression of the other reported CCL28 receptor CCR3, and IgA ASC accumulation in the lactating mammary gland requires CCL28-mediated interactions (7). Using CCR10-deficient mice, we demonstrate that CCR10 interactions are indispensable for efficient accumulation of high levels of IgA ASC in the lactating mammary gland. Conversely, homing of IgA ASC to the large intestine, a site of abundant CCL28 expression, is minimally impacted in CCR10"/" mice. The use of CCR10-deficient mice will allow characterization of the role of this chemokine receptor in other immune functions, as well as in models of inflammation and disease.

Materials and Methods

Construction of the EGFP-containing targeting vector

A pBluescript SK" plasmid containing a 5.8-kb murine CCR10 genomic DNA clone (18) was used to generate the targeting construct. The existing EcoRV site was removed using EcoRV and ClaI, and the first ATG codon of CCR10 exon I was mutated into a new EcoRV restriction site via PCR-directed mutagenesis (QuickChange site-directed mutagenesis kit; Stratagene). The enhanced GFP (EGFP) gene (including its SV40 poly(A) sequence, and excluding its CMV promoter) was excised from a pEGFP-N1 plasmid using XhoI and EcoRI, and inserted into a pBluescript SK" plasmid. The Neo" gene was removed from a pPNT plasmid using NotI and EcoRI and inserted into the EGFP-containing pBluescript SK" plasmid immediately downstream from EGFP to create a 1.8-kb EGFP/NeoR cassette. The plasmid containing the mutated CCR10 linearized with EcoRV, and the EGFP/NeoR cassette was excised using XhoI and BamHI and introduced at the mutated ATG site of CCR10 to generate the targeting construct (Fig. 1A).

Targeting of the CCR10 gene in ES cells and generation of CCR10-deficient mice

The targeting construct for mouse CCR10 was excised using KpnI and XhoI and subcloned into a pPNT plasmid for positive/negative selection with neomycin and gancyclovir. The targeting vector was linearized with KpnI, and introduced into murine J1 embryonic stem (ES) cells by electroporation (−102 cells, 16 μg targeting vector, 400 V at 25 μF). Targeted ES cells were submitted to positive/negative selection, and homologous recombinants were confirmed by Southern blot and injected into blastocysts of C57BL/6 mice to generate chimeric mice for germline transmission. Homozygous mice were bred with wild-type BALB/c mice to give seventh generation BALB/c backcrossed mice. Resulting heterozygous mice were bred against each other to yield the homozygous CCR10"/" and CCR10"/" mice used in our studies. Mice were genotyped by Southern blot analysis of tail genomic DNA digested with XciI and hybridized with a 32P-labeled 350-bp external probe (Fig. 1B).

RNA isolation

Tissues were collected and stored in RNAlater (Ambion) overnight at 4°C. Tissues were then homogenized, and the homogenates were transferred to Nucleospin filter units (BD Clontech) and spun at 14,000 rpm for 3 min. Tissues were then homogenized, and the homogenates were transferred to Nucleospin filter units (BD Clontech) and spun at 14,000 rpm for 3 min.

RT-PCR

RT-PCR was performed using the One Step RT-PCR kit (Qiagen). The primer used for amplification of wild-type CCR10 was 5'-CGGAGAAACCCTTGTAGCCAG-3' (ATG codon): 5'-CGGAGAAACCCTTGTAGCCAG-3'. The sense primer was designed 215 bp downstream from the sense primer: 5'-GGCC AAGACTAGGCGATGC-3'. RNA was reverse transcribed for 30 min and then amplified as described above. In all reactions, potential contamination by genomic DNA was determined using no reverse transcriptase controls. All primers were synthesized by Integrated DNA Technologies.

Relative quantitative PCR

Distal ileum (small intestine) and proximal colon (large intestine) tissue samples were collected from CCR10"/" and CCR10"/" mice. In our collection of small intestine samples, we performed a careful visual analysis of the tissue, inspecting all faces for Peyer’s patches. Visible Peyer’s patches were removed and discarded before further analysis of the ileal tissue. RNA was collected from these samples using TRIzol reagent, according to manufacturer’s instructions (Invitrogen). Quantitative PCR was performed in duplicate on each sample using a Verso 1-step qRT-PCR ROX kit (Thermo Fisher Scientific). GAPDH-specific primers with VIC-MGB probe were used as an endogenous control. Primer and probe for the IgA C region were designed and manufactured by Applied Biosystems. IgA C region primer forward primer (5'-AATCCATATAGGGAATCATTAAACTG-3') and reverse primer (5'-GCGCCACCAAGGAGATGAACTG-3') were used in conjunction with FAM-labeled probe. Quantitative PCR assays were performed on an Applied Biosystems 7300 Real-Time PCR System, and the results were analyzed using Relative Quantification software from Applied Biosystems. Briefly, this software is designed to generate amplification curves of an endogenous control (GAPDH). These values are then used to standardize any differences in RNA concentrations between samples. Levels of IgA mRNA expression from each sample were then assessed by assigning each individual CCR10"/" mouse RNA sample to an arbitrary value of 1 and then comparing each CCR10"/" mRNA sample with this calibrator sample. This process was repeated for each CCR10"/" sample, and resultant values were averaged. A numerical value for each CCR10"/" RNA sample was then generated that represents the fold increase or decrease of IgA mRNA when compared with the appropriate calibrator sample (CCR10"/" mouse large or small intestine).

Immunohistology

Mammary gland tissue was collected from mice 9 days postpartum and frozen in Tissue-Tek OCT mounting medium. Frozen sections (8 μm) were fixed in cold acetone for 10 min. After drying, slides were stained using FITC-labeled anti-IgA, PE-labeled anti-CD45R, and allophycocyanin-labeled anti-TCRβ (both from BD Biosciences). In some experiments, counter staining of nuclei was performed using the DNA binding stain TO-PRO-3 (Invitrogen). Staining was visualized using confocal microscopy. IgA-staining lymphocytes were counted by photographing random mammary gland sections and visually analyzing photographs for the number of stained cells/field of view. Data are expressed as mean ± SEM. Multiple tissue sections from five mice were examined from CCR10"/" and CCR10"/" groups.

Cell isolation and ELISPOT analysis of mouse tissues

IgA ASC numbers in the mammary gland of lactating mice were determined by analysis of lymphocytes in the fourth abdominal mammary gland for all experiments. Mammary gland lymphocytes were isolated by collagenase digestion of 0.1 g mammary gland tissue after removal of the submammary lymph node. All tissues were harvested 9 days postpartum. IgA ASC were identified by ELISPOT analysis of collagenase-digested tissue homogenates. Briefly, Multiscreen 96-well ELISPOT plates (Millipore) were coated with rat anti-mouse IgA (clone C10-3; BD Biosciences). Cell homogenates were then cultured overnight in complete RPMI (RPMI 1640 medium with 10% FBS and 10 U/ml penicillin and streptomycin) in 5% CO2 at 37°C. Plates were then washed repeatedly with HBSS, and captured IgA was detected by the addition of HRP-conjugated goat anti-mouse IgA (Zymed Laboratories), followed by the addition of NovaRed substrate (Vector Laboratories). Addition of the substrate yielded a reddish spot where IgA ASC had secreted Ab during overnight culture. Numbers of IgA ASC/g mammary tissue were then calculated, and statistical significance was determined using Student’s t test. Cell isolation and ELISPOT analysis of intestinal tissues were performed, as previously described (17).

ELISA

For fecal Ig quantification, feces were diluted into a 10% (w/v) PBS solution containing 5% FCS and 0.05% sodium azide, incubated at RT for 1 h, and centrifuged for 2 min at 10,000 rpm. The supernatants were collected and kept at −80°C until ELISA were performed. For serum Ig quantification, blood was collected by cardiac puncture into a Microtainer serum separator tube (BD Biosciences), incubated at room temperature for 30 min, and centrifuged at 8000 rpm for 10 min, and the resulting serum was stored at −80°C. ELISA plates (Nunc) were incubated overnight at 4°C with 100 μl of goat anti-mouse IgA Ab (Bethyl...
FIGURE 1. Inactivation of the CCR10 gene. A, Partial restriction map of the CCR10 gene, the targeting construct, and the inactivated gene after homologous recombination. The probe external to the targeting construct and used to screen ES cells and mice, which distinguishes the 6-kb wild-type and 8.8-kb targeted alleles after SacI digestion, is indicated. B, Southern blot analysis of SacI-digested tail DNA from CCR10+/+, CCR10−/−, and CCR10−/+ mice using the external probe. C, RT-PCR analysis of Peyer’s patch RNA from CCR10+/+, CCR10−/−, and CCR10−/+ mice using CCR10 sense and antisense primers located upstream and downstream, respectively, from the site of the targeting construct. Lanes 1, DNA ladder; lanes 2, 4, and 6, represent no reverse transcriptase reactions used to control for genomic DNA contamination. Lanes 3, 5, and 7, Show PCR products from CCR10+/+, CCR10−/−, and CCR10−/+ mice, respectively.

Laboratories) diluted in sodium phosphate buffer. Wells were washed three times and incubated for 1 h at RT with 200 μl of blocking solution (BD OptiEIA Assay Diluent; BD Biosciences). Wells were again washed three times and incubated for 1 h at RT with 100 μl of sample or known concentration of mouse reference Abs (Bethyl Laboratories). After five washes, wells were incubated for 1 h at RT with 100 μl of goat anti-mouse IgA-HP conjugate (Bethyl Laboratories). Wells were washed five times and incubated with 100 μl of HRP substrate for 20 min. The reaction was stopped with 50 μl of 2 N H2SO4, and the OD was read at 450 nm. Ab concentrations were determined by constructing a standard curve of known values. Data are expressed as mean ± SEM.

Chemotaxis assays

In vitro chemotaxis migration assays were performed, as previously described (17). Briefly, medium alone or medium containing CXCL12 (100 nM), CCL25 (250 nM), or CCL28 (250 nM) was placed in the lower chamber of Transwell chambers (Corning Costar). Splenocytes isolated from CCR10+/+ and CCR10−/+ mice were placed in the upper chamber. Following migration of lymphocytes in response to chemotactic stimuli, cells in the lower chamber were harvested. Phenotypic analysis and quantification of the migration of IgA ASC were determined by flow cytometry, as previously described (7).

Adoptive transfer experiments

In adoptive transfer experiments, CCR10−/+ mothers were injected i.p. with 10° splenocytes from donor mice 1 day postpartum. One group of mice was injected with splenocytes collected from CCR10+/+ mice. As a control, a second group of mice was injected with CCR10−/+ splenocytes. Mammary tissue was harvested from recipient mice 14 days posttransfer, and relative levels of IgA mRNA were determined, as described above.

Animal studies

All mouse studies were performed according to institutional and National Institutes of Health guidelines for animal use and care.

Statistical analysis

Unpaired Student’s t test was used to analyze the results, and p < 0.05 was considered statistically significant.

Results

Generation of CCR10-deficient mice

CCR10-deficient mice were generated by targeted disruption of the first ATG codon of CCR10. Our decision to disrupt the CCR10 gene via insertion only and not via deletion was based on an initial report that deletion in a gene lying in close proximity to CCR10 resulted in severe neurological disorders in mutant mice (19). The correct targeting event was confirmed via Southern blot (Fig. 1B). The insertion of EGFP/NeoR in the CCR10−/− mice by RT-PCR was then confirmed by PCR using a pair of primers upstream and downstream from the ATG codon. RT-PCR resulted in the amplification of a 215-bp sequence from the Peyer’s patch RNA of CCR10+/+ and CCR10−/− mice, and no amplification from the Peyer’s patch RNA of CCR10−/+ mice (Fig. 1C). The absence of CCR10−/− mRNA amplification supports the insertion of the 2.8-kb EGFP/NeoR cassette within the generated EcoRV restriction site located within the start codon of CCR10. In heterozygote mice, coexpression of EGFP with IgA, but not other Ab isotypes was observed. Flow cytometric analysis also demonstrated EGFP expression on other leukocyte subsets, such as neutrophils from the blood and spleen, which have not been shown to express CCR10 (data not shown). These results indicate that EGFP fluorescence on IgA ASC may be an indicator of CCR10 promoter activity. However, “leaky” expression of EGFP on other leukocytes diminishes the utility of relying solely on GFP expression to identify CCR10-expressing cells in heterozygous animals.

FIGURE 2. Splenic IgA ASC from wild-type, but not CCR10 gene-deficient, mice migrate efficiently to the CCR10 ligand CCL28. Chemotaxis of IgA ASC from CCR10−/+ (□) and CCR10−/+ (■) mice was measured in response to select chemokines. Passive chemotaxis was determined by migration to medium alone. Chemokine concentrations were as follows: 100 nM CXCL12, 300 nM CCL25, and 250 nM CCL28. Data demonstrate that the CCR10 gene is functionally deleted in CCR10−/− mice, whereas the chemotactic responses mediated by CCR9 and CXC4 are unaltered. Data represent four mice in each group. **, Indicates statistical significance at p < 0.01 between CCR10−/− and CCR10+/+ groups.
IgA ASC from CCR10-deficient mice do not respond to the CCR10 ligand CCL28

We next sought to confirm, through functional analysis, the absence of target protein in CCR10-deficient mice. In these experiments, we used Transwell migration assays in which the percentage of IgA ASC chemotaxis in response to CXCR4, CCR9, and CCR10 ligands was determined. Analysis of IgA ASC migration showed no statistical differences in CXCR4/CXCL12 or CCR9/CCL25 interactions of IgA ASC isolated from the spleen of CCR10<sup>+/−</sup> and CCR10<sup>+/+</sup> groups were not observed.

FIGURE 3. Fecal and serum levels of IgA Ab are statistically similar in wild-type and CCR10-deficient mice. Fecal and serum Ab levels were measured by sandwich ELISA and displayed as µg IgA/g feces (A) or as µg IgA/ml serum (B). Data represent five mice per group. Statistically significant differences between CCR10<sup>−/−</sup> and CCR10<sup>+/+</sup> groups were not observed.

IgA ASC from CCR10-deficient mice do not respond to the CCR10 ligand CCL28

Following the generation of CCR10-deficient mice, we sought to determine whether they had normal levels of IgA Ab. The Peyer’s patch is a major site of IgA class switching, and the CCR10 ligand CCL28 has been shown to be expressed in the Peyer’s patch (20). Therefore, we addressed the possibility that CCR10/CCL28 interactions are important in the generation of normal levels of IgA ASC, through the analysis of total IgA levels in serum and feces. Both CCR10<sup>+/+</sup> and CCR10<sup>−/−</sup> mice had similar levels of IgA Ab in serum and feces, suggesting there is no overt defect in the generation of IgA ASC in CCR10<sup>−/−</sup> mice (Fig. 3).

CCR10 deficiency results in no reduction of IgA ASC in the small or large intestine

IgA ASC have previously been shown to exhibit robust migration to the CCR9 and CCR10 ligands CCL25 and CCL28, respectively (17, 21). Although both chemokines are expressed in the gastrointestinal tract, they exhibit distinct expression patterns, with CCL25 being expressed at high levels in the small intestine and CCL28 being expressed abundantly in the large intestine and at lower levels in the small intestine (14, 22). This differential expression of CCL25 and CCL28 has led to the hypothesis that CCR9 mediates IgA ASC homing to the small intestine, whereas CCR10 participates in IgA ASC homing to the large intestine. This is supported by a study showing that CCL25 blockade reduces IgA ASC accumulation to the small intestine, whereas CCL28 blockade inhibits accumulation to both small and large intestine (13).

FIGURE 4. CCR10-deficient mice exhibit normal levels and localization of IgA ASC in the small and large intestine. A, ELISPOT analysis of collagenase-digested small and large intestine was performed to determine whether IgA ASC accumulation was inhibited in CCR10<sup>−/−</sup> mice (■) in relation to CCR10<sup>+/+</sup> mice (▲). The y-axis represents numbers of IgA ASC X10<sup>5</sup> tissue. B, Relative quantification of IgA C region mRNA from small and large intestine (■, CCR10<sup>−/−</sup> animals; ▲, CCR10<sup>+/+</sup> animals). The y-axis represents fold IgA mRNA difference between groups with CCR10<sup>+/+</sup> mRNA values standardized to a value of 1. No statistically significant differences in the numbers of IgA ASC or IgA mRNA were detected in the small or large intestine of CCR10<sup>−/−</sup> mice in relation to CCR10<sup>+/+</sup> mice. Data generated from five to eight mice from each group. C–F, Immunohistologic staining of IgA ASC in the gastrointestinal tract of CCR10<sup>+/+</sup> and CCR10<sup>−/−</sup> animals. C and D, Distal ileum from CCR10<sup>+/+</sup> and CCR10<sup>−/−</sup> animals, respectively. E and F, Colon from CCR10<sup>+/+</sup> and CCR10<sup>−/−</sup> animals, respectively. IgA ASC (green); nuclei (blue).
Additionally, CCR9-deficient mice show a ~50% reduction in the number of IgA ASC in the intestinal villi (23). To assess the importance of CCR10 in lymphocyte accumulation, we determined the numbers of IgA ASC in the small and large intestines of CCR10-deficient and wild-type mice through ELISPOT analysis. In our analyses, we observed no statistically significant changes in the IgA ASC levels of the small or large intestine (Fig. 4A). We next confirmed our ELISPOT results by quantitative PCR of mRNA collected from the terminal ileum (small intestine) and the proximal colon (large intestine) using primers and probe specific for IgA C region. In support of the ELISPOT data, a comparison of IgA C region mRNA from the large and small intestine showed no statistical differences in IgA mRNA expression between gene-deficient and wild-type mice (Fig. 4B). Although a trend toward higher levels of IgA mRNA was observed in the ileum of CCR10-deficient mice, this trend was not statistically significant. Immunohistological comparison of IgA ASC localization showed no apparent differences between IgA ASC localization in the gastrointestinal tissues from CCR10+/+ and CCR10−/− mice (Fig. 4, C–F). These results suggest that although the CCR10 chemokine receptor may contribute to IgA ASC localization (13), it is not required for efficient accumulation or tissue localization of IgA ASC in the murine gastrointestinal tract.

**CCR10 deficiency reduces IgA-secreting plasma cell accumulation in the lactating mammary gland**

IgA ASC homing to the murine mammary gland has been shown to be highly dependent on the expression of the CCL28, whereas CCL25 appears to play a minimal, if any, role in the homing of IgA ASC to this tissue (7) (E. Wilson, unpublished results). Immunostaining of mammary gland tissues from lactating mice (9 days postpartum) showed a dramatic reduction in the number of IgA ASC accumulating in the mammary gland of CCR10−/− mice (Fig. 5, A and B). Enumeration of fluorescently stained IgA ASC showed a statistically significant, dramatic reduction in the number of IgA ASC accumulating in the mammary gland of CCR10−/− mice compared with wild-type mice (Fig. 5C). IgA ASC levels in the mammary gland of CCR10-deficient and wild-type mice were confirmed by ELISPOT analysis of collagenase-digested mammary tissues and showed a greater than 70-fold decrease (p < 0.01) in the number of IgA ASC in the mammary gland of lactating wild-type mice compared with CCR10−/− mice (Fig. 5D). To distinguish whether the observed effect of CCR10 deficiency on the accumulation of IgA ASC into the mammary gland was due to the inability of IgA ASC to efficiently migrate to this tissue, or due to secondary effects of the knockout on mammary gland development, we performed adoptive transfer experiments in which splenocytes from wild-type donor mice were adoptively transferred into CCR10−/− recipients. The control group for this experiment consisted of CCR10−/− mice adoptively transferred with CCR10+/+ splenocytes. Analysis of these experiments demonstrated that CCR10+/+ ASC efficiently accumulate in the mammary tissues of CCR10−/− animals (Fig. 5E). These results suggest that the lack of IgA ASC accumulation in the mammary gland of CCR10−/− animals is a result of a lack of CCR10 expression and not due to abnormal mammary gland development resulting from a lack of CCR10 expression.

**CCR10 deficiency impairs IgA accumulation in the milk and immune transfer to the neonate**

IgA ASC accumulation in the lactating mammary gland ultimately leads to the deposition of large quantities of IgA in the milk. This milk-borne IgA is passively transferred to the gastrointestinal tract of the nursing neonate, where it provides immunoprotection. IgA
levels in the milk of lactating mice were significantly \((p < 0.01)\) reduced in CCR10\(^{-/-}\) vs CCR10\(^{+/+}\) mice (Fig. 6A). Based on the dramatically reduced levels of IgA found in the milk of CCR10-deficient mothers, we predicted that CCR10 deficiency in lactating mice would lead to diminished passive transfer of Ab to the neonate. To assess this, we measured the concentrations of IgA in the feces of 9-day-old pups. Differences observed in A and B are statistically significant \((p < 0.01)\).

Discussion

The homing of lymphocytes depends on multiple molecular interactions occurring in a sequential order (24). Interactions between tissue-expressed chemokines and leukocyte-expressed chemokine receptors have been shown to up-regulate integrin affinity, resulting in the firm adhesion of lymphocytes to cellular adhesion molecules expressed on endothelial cells (25–27). Chemokines are also thought to mediate transendothelial migration, lymphocyte locomotion, and retention in distinct microenvironments within a tissue (28–35).

The chemokine receptor CCR10, which binds CCL28 and CCL27, has been suggested to play a major role in vivo lymphocyte trafficking to portals of microbial entry, such as mucosal tissues and the skin. CCL28 has been shown to be involved in IgA ASC homing to the gastrointestinal tract and lactating mammary gland and hypothesized to play a role in T regulatory accumulation to mucosal epithelium (7, 13, 36). The CCR10 ligand CCL27 has been suggested to be an important mediator of leukocyte homing and accumulation to the skin for a variety of cells, including keratinocytes and effector/memory T cells (9, 37, 38). Although in vivo experimentation has effectively demonstrated the role of CCL27 and CCL28 in lymphocyte trafficking, the role of CCR10 has not previously been shown in vivo. In this study, we show, through the deletion of functional CCR10, that this chemokine receptor is vital to the efficient accumulation of IgA ASC to the lactating mammary gland.

In this study, our aim was to understand the role of CCR10 in the localization of IgA ASC in vivo. Our results clearly show that CCR10-mediated interactions are imperative for efficient IgA ASC accumulation into the mammary gland. This finding is consistent with and extends our earlier study of the role of CCL28 in IgA ASC-homing lactating mammary gland (7).

In assessing the role of CCR10-mediated accumulation to the gastrointestinal tract, we assayed total IgA ASC numbers as well as IgA protein and mRNA levels. Conversely, previous work that showed a role for CCL28 in mediating intestinal accumulation of IgA ASC focused on cholera toxin-specific cells (13). This methodological difference may be significant in that the IgA Ab response to cholera toxin has been shown to be strongly CD4 T cell dependent (39). Yet, the gut lamina propria has been shown to be populated by significant numbers of IgA ASC that originate in the absence of T cells, as well as in the absence of Peyer’s patches and mesenteric lymph nodes (40–42). These results raise the possibility that IgA ASC generated by T cell-dependent class switching may use CCR10 and home efficiently to the mammary gland and gastrointestinal tract. Conversely, IgA ASC generated by T cell-independent mechanisms may be less reliant on CCR10-mediated interactions, yet efficiently homed in large numbers to the gastrointestinal tract via other mechanisms.

There is strong evidence that CCL25/CCL28-independent pathways can participate in IgA ASC localization to intestinal tissues. Functional blockade of CCL25 and CCL28 was reported to inhibit IgA ASC accumulation in the gastrointestinal tract by only ~50% (13). In agreement with that result, a study using a neonatal mouse model of gastrointestinal infection showed that functional blockade of CCL25 and CCL28 leads to only partial reduction of IgA ASC accumulation into the gastrointestinal tract (11). These two studies suggest that CCL25 and CCL28 play important, yet clearly nonexclusive roles in the accumulation of IgA ASC into mucosal tissues. Other chemokines involved in the accumulation of IgA ASC to mucosal tissues remain to be identified.

The efficient migration of IgA ASC to mucosal tissues is vital to protecting the host from reinfection with gastrointestinal pathogens. The common mucosal immune system has been hypothesized to maximize IgA ASC recruitment to mucosal tissues (43), and has more recently been suggested to comprise both common and regional components (44, 45). The potential for regionalization within the mucosal immune system is illustrated by the differential expression of the IgA ASC-attracting chemokines CCL25 and CCL28 in the small and large intestines, respectively. Data presented in this study demonstrate that CCR10-mediated interactions are of varying importance in the accumulation of IgA ASC in the mammary gland and large intestine, two tissues that express high levels of CCL28. These findings demonstrate a higher degree of regionalization within the mucosal immune system than was previously appreciated.

In summary, our data effectively demonstrate the indispensable nature of the chemokine receptor CCR10 in maintaining normal levels of IgA ASC in the lactating mammary gland, but not the large intestine. These findings, taken together with our previous results, indicate that CCR10/CCL28 interactions are required for ASC homing to the mammary gland. The disparate levels of IgA ASC accumulation in different mucosal tissues, such as the small and large intestines and mammary gland of CCR10\(^{-/-}\) vs CCR10\(^{+/+}\) mice, indicate that CCR10-mediated homing of IgA ASC is not of uniform importance in all CCL28-expressing regions of the mucosal immune system. These data support the concept of significant regionalization within the common mucosal immune system.

FIGURE 6. Passive immune transfer of IgA to the nursing neonate is inhibited in CCR10\(^{-/-}\) mice. A, IgA Ab levels in the milk 9 days postpartum of lactating CCR10\(^{-/-}\) mothers compared with CCR10\(^{+/+}\) mothers. B, IgA Ab levels in the feces of 9-day-old pups. Differences observed in A and B are statistically significant \((p < 0.01)\).
Disclosures
The authors have no conflicting financial interests.

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