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A Role for CCR9 in T Lymphocyte Development and Migration

Shoji Uehara,* Alexander Grinberg,* Joshua M. Farber, † and Paul E. Love²*

CCR9 mediates chemotaxis in response to CCL25/thymus-expressed chemokine and is selectively expressed on T cells in the thymus and small intestine. To investigate the role of CCR9 in T cell development, the CCR9 gene was disrupted by homologous recombination. B cell development, thymic αβ-T cell development, and thymocyte selection appeared unimpaired in adult CCR9-deficient (CCR9<sup>−/−</sup>) mice. However, competitive transplantation experiments revealed that bone marrow from CCR9<sup>−/−</sup> mice was less efficient at repopulating the thymus of lethally irradiated Rag<sup>1−/−</sup> mice than bone marrow from litterate CCR9<sup>+/+</sup> mice. CCR9<sup>−/−</sup> mice had increased numbers of peripheral γδ-T cells but reduced numbers of γδTCR<sup>+</sup> and CD8αβ<sup>+</sup> αβTCR<sup>+</sup> intraepithelial lymphocytes in the small intestine. Thus, CCR9 plays an important, although not indispensable, role in regulating the development and/or migration of both αβ<sup>−</sup> and γδ<sup>−</sup> T lymphocytes. The Journal of Immunology, 2002, 168: 2811–2819.
important role in regulating the development and/or migration of γδ-T lymphocytes.

**Materials and Methods**

**Generation of CCR9-deficient mice**

An ~15-kb fragment containing the mouse CCR9 gene was cloned from a 129 SvJ Å genomic library (Stratagene, La Jolla, CA). A 7-kb EcoRV fragment and a 1.6-kb HindIII-EcoRV fragment were then cloned into the XpPNT (NEO/TK) vector (24). The targeting construct was linearized with NotI and electroporated into 2 × 10⁷ embryonic stem cells (ES cells). After transfection, ES cells were selected in the presence of G418 and gancyclovir and screened for homologous recombination. Chimeric mice were generated from CCR9°/° ES cell clones by injection into B6 blastocysts, and germline transmission of the mutant allele was confirmed by Southern blot analysis of DNA obtained from tail biopsies.

H-Y (25), P14 (26), and AND (27) αβTCR transgenes were bred into the CCR9°/° background. The phenotype of thymocytes and peripheral T cells was analyzed by staining with PE-labeled anti-CD4, CyChrome-labeled anti-CD8α, and FITC-labeled anti-H-Y clonotypic receptor (T3.70), anti-TCR-Vα2 (P14), or anti-TCR-Vα11 (AND) mAb as previously described (28). Mice were maintained in the H-2Dk background by mating with C57BL/6 mice.

**Cell preparation and flow cytometry**

Thymus, spleen, lymph node, and bone marrow were excised from mice, and single-cell suspensions were prepared. IEL were prepared from small and large intestines according to conventional methods (29). Standard cytometry was performed as previously described using a FACSCalibur and CellQuest software (BD Biosciences, Mountain View, CA) (21). Anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CD19, anti-DX5, and anti-TCR-Vα11 (AND) mAbs were purchased from BD PharMingen (San Diego, CA). The polyclonal anti-CCR9 Ab has been described previously (21). Anti-H-Y clonotypic receptor mAb (T3.70) was purified from cell culture supernatants in our laboratory. Anti-TCR-Vα2, anti-TCR-Vα11, and anti-DX5 mAb were purchased from BD PharMingen (San Diego, CA). The monoclonal anti-CCR9 Ab has been described previously (21). Anti-H-Y clonotypic receptor mAb (T3.70) was purified from cell culture supernatants in our laboratory. Anti-TCR-Vα11 (32) and anti-TCR-Vγ2 (30) mAbs were provided by Dr. L. Lefrancois (University of Connecticut Health Center, Farmington, CT). Unconjugated anti-FcγRII (2.4G2) was used to block nonspecific binding of the labeled Ab. PE-conjugated streptavidin and CyChrome-conjugated streptavidin were also purchased from BD PharMingen.

**Chemotaxis assays**

Chemotaxis assays were performed as previously described (18, 21) with modifications, using 6.5-mm Transwell tissue culture inserts with a 5-μm pore size (Corning, Cambridge, MA). Thymocytes were suspended at 1 × 10⁷ cells/ml in RPMI 1640 plus 0.5% BSA, and 100 μl cell suspension was added to an insert in a well with 600 μl medium. After equilibration at 37°C for 1 h chemokines were added to the wells and the plates were incubated for an additional 2 h before cells were harvested, collected by centrifugation, and counted. Duplicate wells were used for each condition. Murine CXCL12 and CCL25 were obtained from PeproTech (Rocky Hill, NJ) and R&D Systems (Minneapolis, MN), respectively.

**Northern blotting**

Total RNA was isolated from various organs from B6 and Rag-1−/− mice using TRisol (Life Technologies, Gaithersburg, MD). Twenty micrograms of total RNA was fractionated on a 1% agarose/formaldehyde gel and transferred onto a GeneScreen Plus nylon membrane (New England Nuclear, Boston, MA). The membrane was hybridized with 32P-labeled cDNA fragments encoding mouse CCR9, mouse CCL25, or human EF-1α.

**Bone marrow chimeras**

Bone marrow cells were isolated from femurs of CCR9°/° (CD45.2), CCR9°/− (CD45.2), and B6.SJL-Ptprcϕ/BoAiTac mice (B6.CD45.1; Tacconic Farms, Lexington, KY). A total of 2 × 10⁶ cells consisting of various ratios of CCR9°/° (CD45.2) and B6.CD45.1 bone marrow cells or CCR9°/− (CD45.2) and B6.CD45.1 bone marrow cells were injected i.v. into lethally irradiated (9.5 Gy) Rag-1−/− B6 mice (CD45.2). One to 2 mo after the transplantation thymocytes and lymph node B cells were analyzed by FACS for the presence of CD45.2+ cells.

**Statistical analysis**

Data from mice of the different phenotypes were analyzed using Student’s t test.

**Results**

**Generation of CCR9-deficient mice**

To investigate the biological significance of CCR9 in vivo, CCR9°/− mice were generated by homologous recombination. The gene-targeting vector was designed to delete exon 4, which contains most of the CCR9-coding sequence (Fig. 1A). The expected structure of the targeted CCR9 locus was confirmed by Southern blotting with the indicated 3′ probe (Fig. 1B). CCR9 mRNA was undetectable in the thymus, spleen, lymph node, and small intestine from CCR9°/− mice (data not shown). FACS analysis confirmed that thymocytes from CCR9°/− mice lacked CCR9 surface expression (Fig. 1C). CCR9°/− mice did not exhibit any developmental abnormalities, were produced in Mendelian ratios,
and were indistinguishable from CCR9+/+ or CCR9+/- littersmates on the basis of size, activity, or fertility (data not shown).

**Normal αβ-lineage T cell development in adult CCR9−/− mice**

We initially focused our analysis on T cell development in CCR9−/− mice, because CCR9 is expressed on the surface of most thymocytes, especially CD4+CD8− (DP) cells (20, 21). The numbers and distribution of the major thymic and peripheral T cell subsets, as defined by the expression of CD4 and CD8, were normal in CCR9−/− mice (Fig. 2). In addition, surface expression of CD3, βTCR, CD5, and CD69 were indistinguishable on thymocytes and lymph node T cells from CCR9−/− and CCR9+/- littersmates (data not shown). Histological studies revealed normal thymus architecture in CCR9−/− mice (data not shown). Peripheral T cells from CCR9−/− mice demonstrated normal proliferative responses after CD3 cross-linking, and CCR9−/− mice had normal Ab responses to T cell-dependent Ags (data not shown). Finally, there were no differences in the number or phenotype of thymocytes from newborn CCR9−/− and CCR9+/- mice (data not shown). Collectively, these data indicate that αβ-T cells can develop normally in the absence of CCR9.

We next analyzed the responsiveness of thymocytes to CCL25. CCR9−/− thymocytes did not respond to CCL25, although they migrated to CXCL12 in a manner comparable to CCR9+/- cells (Fig. 3). CCR9+/- thymocytes expressed CCR9 at lower levels than CCR9+/- littersmates (Fig. 1C) and exhibited a reduced chemotactic response to CCL25 (Fig. 3). These data indicate that CCR9 expression is essential for chemotaxis of thymocytes to CCL25. CCL25 preferentially induces the migration of CD3+CD69+DP thymocytes compared with CD69−DP thymocytes (20, 22). TCR cross-linking of DP thymocytes enhances CCL25-mediated migration (21). These data suggest that CCL25 may be involved in thymocyte selection. To determine whether positive or negative selection was affected in CCR9−/− mice, we crossed the H-Y TCR transgene into the CCR9−/− background and analyzed the phenotype of thymocytes in the positively selecting (female) or negatively selecting (male) background. The absence of CCR9 did not alter the efficiency of either positive or negative thymocyte selection in H-Y TCR transgenic mice (data not shown). In addition, no differences in the efficiency of thymocyte selection were observed in CCR9−/− mice that expressed the P14-TCR transgene or the AND-TCR transgene (data not shown). These data suggest that CCR9 does not play a critical role in thymocyte selection.

**FIGURE 3.** Lack of chemotactic response of thymocytes from CCR9−/− mice to CCL25. Total thymocytes from CCR9+/-, CCR9−/−, and CCR9+/- mice were used for chemotaxis assays with CCL25 (200 nM) and CXCL12 (50 nM). Results are expressed as cells migrating per 10^6 input cells. Determinations were performed in duplicate, and error bars represent the SD.

Approximately half of all γδTCR+ cells in thymus, spleen, and lymph node express CCR9 and can respond to CCL25 (21). Therefore, we next compared the number and phenotype of γδ-T cells in different organs from CCR9−/− and CCR9+/- mice. The number and percentage of γδTCR+ thymocytes were similar in CCR9−/− and CCR9+/- mice (Fig. 4A). However, both the absolute number and the percentage of γδTCR+ cells were increased 2-fold in spleen and lymph nodes of CCR9−/− mice compared with CCR9+/- mice (Fig. 4A). We previously observed that γδTCR+ cells that resemble recent thymic emigrants (i.e., CD44lowCD5RB+B220+) preferentially express CCR9 (21). However, no significant differences in CD44, CD45RB, CD62L, or αhel integrin expression were observed on peripheral γδ-T cells from CCR9−/− and CCR9+/- mice (data not shown). To ascertain...
from CCR9−/− mice resembled those from CCR9+/+ mice in that they were uniformly CD8α− and expressed high levels of αEL integrin (data not shown).

Although the TCR-Vγ/δ repertoire is diverse in γδTCR+ IEL, the γδTCRs expressed by small intestinal IEL consist predominantly of TCR-Vγ1 or -Vγ5 paired with TCR-Vδ4, -Vδ5, -Vδ6, or -Vδ7 (33, 34). To assess whether there were any differences in the TCR-Vγ/δ repertoire of γδTCR+ IEL in small intestine, we analyzed γδTCR+ IEL in CCR9−/− and CCR9+/+ mice for the expression of TCR-Vγ1, -Vγ2, -Vγ5, and -Vδ4 by flow cytometry. The percentages of TCR-Vγ1, -Vγ2, and -Vγ5-bearing cells among γδTCR+ IEL were similar in CCR9−/− and CCR9+/+ mice (Fig. 5, D and E). However, the percentage of TCR-Vδ4-bearing IEL was markedly decreased in CCR9−/− mice (Fig. 5, D and E). Most TCR-Vδ4+ IEL coexpress TCR-Vγ5 and, to a lesser extent, TCR-Vγ1. Consistent with this observation, both TCR-Vγ5/Vδ4+ and TCR-Vγ1/Vδ4+ IEL subsets were decreased in CCR9−/− mice (Fig. 5F). Collectively, these data indicate that CCR9 is involved in the generation or maintenance of γδTCR+ IEL and, in particular, TCR-Vδ4+ IEL in small intestine.

We next evaluated αβTCR+ IEL in the small intestine of CCR9−/− and CCR9+/+ mice. αβTCR+ IEL subsets can be distinguished by the expression of CD8α, CD8β, and CD4. The percentage of αβTCR+ IEL that were CD4−CD8α− and CD4+CD8β− was not consistently different in CCR9−/− and CCR9+/+ mice (Fig. 5F). However, the percentage of CD8αβ+ IEL was reduced in CCR9−/− mice (Fig. 5F). No significant differences were observed in the number or subset distribution of small intestinal lamina propria lymphocytes in CCR9−/− and CCR9+/+ mice (data not shown).

Normal B lymphopoiesis in CCR9−/− mice

Northern blot analysis revealed that CCR9 mRNA is expressed in bone marrow from B6 mice (Fig. 6A). Rag-1−/− bone marrow cells, which lack mature T and B cells, had an equivalent level of CCR9 mRNA expression, indicating that CCR9 is expressed on lymphoid progenitor cells and/or myeloid cells. In contrast to CCR9 expression, CCL25 mRNA was undetectable in the bone marrow (Fig. 6A). Bowman et al. (23) described an immature population of bone marrow cells that migrates in response to CCL25. This population is phenotypically similar to DX5+ CD19+ B220+ bone marrow cells (35, 36). DX5−CD19− B220− cells can be further subdivided on the basis of CD4 surface expression into B cell precursors (CD4+ DX5+ CD19− B220+) and cells of unknown lineage and potential (CD4− DX5+ CD19− B220+) (37). Significantly, staining of bone marrow cells with anti-CCR9 Ab revealed high level expression of CCR9 on CD4+DX5+ CD19− B220+ cells, but only very low levels of CCR9 on CD4− DX5− CD19− B220+ cells (Fig. 6B). Both populations migrated in response to CCL25 (data not shown). No statistically significant differences in the number or the percentage of CD4+ DX5+ CD19− B220+ and CD4− DX5− CD19− B220+ bone marrow cells were observed in CCR9−/− and CCR9+/+ mice (Fig. 6C). In addition, CCR9−/− mice contained normal numbers of bone marrow cells and peripheral B cells and contained normal proportions of pro-B (CD43+ IgM− B220+), pre-B (CD43+ IgM+ B220+), immature B (CD43− IgM− B220−), and recirculating bone marrow (CD43+ IgM+ B220+)' B cells and exhibited normal IgM and IgD surface profiles on splenic B cells (Fig. 6C and data not shown). Thus, CCR9 is not essential for normal B cell development.

Abnormal distribution of γδTCR+ IEL in CCR9−/− mice

Mucosal lymphocytes are composed of IEL and lamina propria lymphocytes. IEL consist of αβTCR+ and γδTCR+ cells, with γδTCR+ cells making up ~50% of the total population in the small intestine and ~20% of the total population in the large intestine (32). CCL25 and CCR9 mRNAs are detectable in duodenum and small intestine, but not in esophagus, stomach, appendix, and large intestine (Fig. 5A). In addition, CCR9 mRNA is expressed in both αβTCR+ and γδTCR+ small intestinal IEL (21). The number of small intestinal IEL or large intestinal IEL was not statistically different in CCR9−/− and CCR9+/+ mice (data not shown). However, in CCR9−/− mice the percentage of γδTCR+ IEL was decreased in small intestine but increased in large intestine (Fig. 5, B and C). There was no difference in CD2 and CD8α expression on large intestinal IEL from CCR9−/− and CCR9+/+ mice (data not shown). In addition, small intestinal γδTCR+ IEL whether γδ-T cell subsets were different in CCR9−/− and CCR9+/+ mice, we analyzed the percentages of TCR-Vγ1, -Vγ2, -Vγ5, or -Vδ4-expressing cells among total CD3+ T cells. TCR-Vγ1+, -Vγ2+, -Vγ5+, or -Vδ4+ cells appeared to be increased in the lymph node (Fig. 4B) and spleen (data not shown) of CCR9−/− mice. On average, TCR-Vγ1+ , TCR-Vγ5+ , and TCR-Vδ4+ cells were increased more than TCR-Vγ2+ cells (TCR-Vγ1+, 3.1-fold; TCR-Vγ2+, 1.8-fold; TCR-Vγ5+, 2.9-fold; TCR-Vδ4+, 2.6-fold). In contrast, no significant difference in TCR-Vγ1-, -Vγ2-, -Vγ5-, or -Vδ4 usage was detected in thymocytes from CCR9−/− and CCR9+/+ mice (data not shown).

Abnormal distribution of γδTCR+ IEL in CCR9−/− mice

Mucosal lymphocytes are composed of IEL and lamina propria lymphocytes. IEL consist of αβTCR+ and γδTCR+ cells, with γδTCR+ cells making up ~50% of the total population in the small intestine and ~20% of the total population in the large intestine (32). CCL25 and CCR9 mRNAs are detectable in duodenum and small intestine, but not in esophagus, stomach, appendix, and large intestine (Fig. 5A). In addition, CCR9 mRNA is expressed in both αβTCR+ and γδTCR+ small intestinal IEL (21). The number of small intestinal IEL or large intestinal IEL was not statistically different in CCR9−/− and CCR9+/+ mice (data not shown). However, in CCR9−/− mice the percentage of γδTCR+ IEL was decreased in small intestine but increased in large intestine (Fig. 5, B and C). There was no difference in CD2 and CD8α expression on large intestinal IEL from CCR9−/− and CCR9+/+ mice (data not shown). In addition, small intestinal γδTCR+ IEL
were observed in adult CCR9 -/ mice. Cells failed to migrate in response to CCL25 (21) (data not shown). These CD4 -/ CD8 -/ CD4 -/ TCR-V /- mice were gated and analyzed for CD3 and CD8 expression on immature thymocyte populations from adult mice. Bleul et al. (12) reported that fetal blood prothymocytes (Thy1 -/ ) respond to CCL25. These findings suggest that CCL25/CCR9 may be involved in the migration of prothymocytes into the thymus. To explore this possibility further, we examined CCR9 expression on immature thymocyte populations from adult mice. CCR9 expression was undetectable on CD3 -/ CD4 -/ CD8 -/ (triple-negative (TN)) thymocytes from adult mice, including the most immature (CD44 /CD25 - TN) subset, and adult TN thymocytes failed to migrate in response to CCL25 (21) (data not shown). In addition, the number and distribution of TN thymocyte subsets in CCR9 -/ mice, as defined by the expression of CD44 and CD25, were similar (data not shown). Thymus size and cellularity can be normal even if the number of immature T progenitor cells in the thymus is reduced, presumably because these cells are capable of expanding (1). Consequently, examination of thymocytes in the adult steady state condition may not reveal a potential defect in the progenitor cell population. To determine whether the loss of CCR9 affects the migration of bone marrow progenitor cells into the thymus or the establishment or retention of T progenitor cells in the thymus, we performed a competitive transplantation experiment. Total bone marrow cells from CCR9 -/ mice (CD45.2) and B6.CD45.1 (CCR9 +/ ) were mixed in different ratios and injected into lethally irradiated Rag-1 -/ mice. As a control, identical mixtures of bone marrow cells from littermate CCR9 +/ and B6.CD45.1 mice were injected into irradiated Rag-1 -/ mice. One to 2 mo after the bone marrow transplantation, the number and percentage of CD45.2 -/ thymocytes and peripheral (lymph node) B cells were determined. Significantly, when mixtures of CCR9 -/ and B6.CD45.1 bone marrow were injected, the percentage of thymocytes derived from CCR9 -/ bone marrow cells was consistently lower than the expected ratio (Fig. 7, A and C). In contrast, the percentage of lymph node B cells derived from CCR9 -/ bone marrow cells was consistently close to the expected ratio (Fig. 7D). The reduction in CCR9 -/ bone marrow-derived thymocytes was evident at both the CD44+/CD25 - TN and the CD44 /CD25 - TN stage (Fig. 7B), indicating that cells from CCR9 -/ mice were competitively disadvantaged at or before the CD44+/CD25 - TN stage. Taken together, these data indicate that under competitive
conditions, bone marrow cells from CCR9−/− mice are impaired in their ability to reconstitute T cell, but not B cell, development in irradiated Rag-1−/− mice.

Discussion

In this study we examined the role of CCR9 in T and B cell development by generating CCR9-deficient mice by gene targeting. CCR9 is expressed on the surface of most αβ-lineage thymocytes and approximately half of all γδTCR+ thymocytes and T cells (20, 21). Mucosal T cells also express CCR9, and fetal blood prothymocytes and pre-pro-B cells migrate in response to CCL25 (11–15, 21, 23). Analysis of CCR9−/− mice revealed that 1) αβ-T cells develop normally in adult CCR9−/− mice, but CCR9−/− bone marrow cells exhibit a reduced capacity to repopulate the thymus of irradiated Rag-1−/− mice under competitive conditions compared with CCR9+/+ bone marrow cells; 2) CCR9−/− mice contain increased numbers of peripheral γδ-T cells, but reduced numbers of small intestinal γδTCR+ IEL; and 3) B cell development is unaffected in CCR9−/− mice.

The finding that αβ-T cell development and thymocyte selection appear unperturbed in adult CCR9−/− mice was unexpected given the fact that most thymocytes express high levels of CCR9 and migrate to CCL25. The inability of CCR9−/− thymocytes to respond to CCL25 demonstrates that these cells do not express another receptor for CCL25. Several different chemokines are expressed in the thymus, and it is possible that they may share overlapping targets and therefore compensate for the loss of CCR9. Histological examination of the thymus revealed no obvious abnormalities in CCR9−/− mice; however, more detailed localization studies of specific thymocyte subsets may be required to detect subtle intrathymic migration defects.

Previous results indicate that prothymocytes in fetal blood respond to CCL25 (12). This observation together with the finding that CCL25 is not expressed in bone marrow (Fig. 6A) suggested that CCR9 may participate in the migration of T-progenitor cells from bone marrow to thymus. Although we were unable to detect CCR9 surface expression on immature TN thymocytes from adult mice, and these cells did not respond to CCL25, CCR9+ cells were present in adult bone marrow (Fig. 6, A and B). CD4−DX5−CD19−B220+ bone marrow cells, which include early B progenitors (37, 38), expressed low levels of CCR9 and could respond to CCL25 (Fig. 6B and data not shown). In addition, CD4+DX5−CD19−B220+ cells were found to express high levels of CCR9 and could respond to CCL25. The lineage affiliation and differentiation potential of CD4+DX5−CD19−B220+ cells remain unclear, although phenotypically similar cells from bone marrow that respond to CCL25 contain both B and T cell progenitors (36, 37, 39, 40). To investigate whether CCR9 is involved in regulating the migration of T-progenitor cells to thymus, we performed competitive bone marrow transplantation experiments. The results demonstrated that CCR9−/− bone marrow cells are competitively disadvantaged compared with CCR9+/+ bone marrow cells in their ability to repopulate the thymus of irradiated Rag-1−/− mice (Fig. 7). These findings suggest three nonmutually exclusive possibilities: 1) that CCR9 regulates the generation of prothymocytes in the bone marrow, 2) that CCR9 regulates the migration of prothymocytes into the thymus or their migration or retention within the thymus, and 3) that CCR9 regulates the proliferation of early thymocytes in the thymus. Because immature TN thymocytes do not express surface CCR9 and do not respond to CCL25 (21), it appears unlikely that CCR9 is directly involved in the expansion of early thymocytes. Bleul et al. (12) reported that three chemokines (CCL25, CXCL12, and CCL21) are expressed in the thymic anlage, and both CCL25 and CXCL12 attract fetal blood prothymocytes. Similar to CCR9−/− mice, mice deficient in CXCL12 or its receptor, CXCR4, also showed no obvious abnormality in T cell development (41–43). On the basis of these findings, we speculate that CCR9 in addition to other chemokine receptors such as CXCR4 may play an important and partially redundant role in regulating the migration of prothymocytes into the thymus. Our inability to detect surface expression of CCR9 on immature TN thymocyte subsets is not necessarily in conflict with this idea, as the population of CCR9+ prothymocytes may be extremely small, or CCR9 may be down-regulated when prothymocytes enter the thymus.

γδ-T cell development and/or homeostasis were clearly altered in CCR9−/− mice. Although CCR9−/− mice contained normal numbers of γδTCR+ thymocytes, the number of γδ-T cells was...
injected with mixtures of bone marrow from CCR9/H11001 and B6.SJL-Ptprc<sup>BaAlTac</sup> mice (B6.CD45.1; Taconic Farms). A total of 2 × 10<sup>7</sup> cells consisting of mixtures of CCR9<sup>+/+</sup> or CCR9<sup>−/−</sup> bone marrow with B6.CD45.1 bone marrow was injected i.v. into lethally irradiated (9.5 Gy) Rag-1<sup>−/−</sup> B6 mice (CD45.2). One to 2 mo after transplantation, thymocytes and lymph node B cells were examined for the expression of CD45.2 by FACS. A. Representative FACS plots showing the expression of CD4, CD8α, and CD45.2 on thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with equal numbers of bone marrow cells from CCR9<sup>+/+</sup> and CD45.2<sup>−/−</sup> and B6.CD45.1 mice (upper left panel) or CCR9<sup>−/−</sup> and B6.CD45.1 mice (upper right panel). Histograms show the expression of CD45.2 on total thymocytes. B. Representative FACS plots showing the expression of CD44 and CD25 on gated (CD4<sup>+</sup>CD8<sup>−</sup>CD3<sup>−</sup> (TN)) thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with equal numbers of bone marrow cells from CCR9<sup>+/+</sup> and B6.CD45.1 mice (upper left panel) or CCR9<sup>−/−</sup> and B6.CD45.1 mice (upper right panel). Histograms show CD45.2 expression on CD44<sup>+</sup>CD25<sup>+</sup> or CD44<sup>−</sup>CD25<sup>−</sup> thymocytes. C. Plot of the percentage of CD45.2<sup>+</sup> cells in total thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with the indicated ratios of bone marrow cells. The percentage of thymocytes derived from CCR9<sup>−/−</sup> bone marrow cells was significantly lower than that of CCR9<sup>+/+</sup> bone marrow-derived thymocytes (p < 0.01). D. Plot of the percentage of CD45.2<sup>+</sup> cells in lymph node B cells (B220<sup>+</sup>) from irradiated Rag-1<sup>−/−</sup> mice injected with the indicated ratios of bone marrow cells. Data from mice injected with mixtures of bone marrow from CCR9<sup>+/+</sup> (CD45.2) and B6.CD45.1 mice; ○, data from mice injected with mixtures of bone marrow from CCR9<sup>−/−</sup> (CD45.2) and B6.CD45.1 mice.

FIGURE 7. Competitive bone marrow transplantation into irradiated Rag<sup>−/−</sup> mice. Bone marrow cells were isolated from femurs of CCR9<sup>+/+</sup> (CD45.2), CCR9<sup>−/−</sup> (CD45.2), and B6.SJL-Ptprc<sup>BaAlTac</sup> mice (B6.CD45.1; Taconic Farms). A total of 2 × 10<sup>7</sup> cells consisting of mixtures of CCR9<sup>+/+</sup> or CCR9<sup>−/−</sup> bone marrow with B6.CD45.1 bone marrow was injected i.v. into lethally irradiated (9.5 Gy) Rag-1<sup>−/−</sup> B6 mice (CD45.2). One to 2 mo after transplantation, thymocytes and lymph node B cells were examined for the expression of CD45.2 by FACS. A. Representative FACS plots showing the expression of CD4, CD8α, and CD45.2 on thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with equal numbers of bone marrow cells from CCR9<sup>+/+</sup> and B6.CD45.1 mice (upper left panel) or CCR9<sup>−/−</sup> and B6.CD45.1 mice (upper right panel). Histograms show the expression of CD45.2 on total thymocytes. B. Representative FACS plots showing the expression of CD44 and CD25 on gated (CD4<sup>+</sup>CD8<sup>−</sup>CD3<sup>−</sup> (TN)) thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with equal numbers of bone marrow cells from CCR9<sup>+/+</sup> and B6.CD45.1 mice (upper left panel) or CCR9<sup>−/−</sup> and B6.CD45.1 mice (upper right panel). Histograms show CD45.2 expression on CD44<sup>+</sup>CD25<sup>+</sup> or CD44<sup>−</sup>CD25<sup>−</sup> thymocytes. C. Plot of the percentage of CD45.2<sup>+</sup> cells in total thymocytes from irradiated Rag-1<sup>−/−</sup> mice injected with the indicated ratios of bone marrow cells. The percentage of thymocytes derived from CCR9<sup>−/−</sup> bone marrow cells was significantly lower than that of CCR9<sup>+/+</sup> bone marrow-derived thymocytes (p < 0.01). D. Plot of the percentage of CD45.2<sup>+</sup> cells in lymph node B cells (B220<sup>+</sup>) from irradiated Rag-1<sup>−/−</sup> mice injected with the indicated ratios of bone marrow cells. Data from mice injected with mixtures of bone marrow from CCR9<sup>+/+</sup> (CD45.2) and B6.CD45.1 mice; ○, data from mice injected with mixtures of bone marrow from CCR9<sup>−/−</sup> (CD45.2) and B6.CD45.1 mice.

Image 142x435 to 443x733

increased in secondary lymphoid organs (spleen and lymph nodes; Fig. 4A). All TCR-VγVδ pairs examined were increased in lymph nodes and spleen of CCR9<sup>−/−</sup> mice (Fig. 4B and data not shown). In addition, we could not detect any significant difference in the expression of CD44, CD45RB, and other surface markers (e.g., CD62L and α<sub>p<sub>integrin</sub> on peripheral γδ-T cells from CCR9<sup>−/−</sup> and CCR9<sup>+/+</sup> mice (data not shown), suggesting that the increased number of peripheral γδ-T cells in CCR9<sup>−/−</sup> mice is not due to the accumulation of one particular subpopulation of γδ-T cells. The kinetics of thymocyte development are much more rapid for γδ- than αβ-T cells, and γδ-T cells appear to be dependent on the thymic environment for a relatively brief period during their development (44). Thus, in the absence of CCR9, γδ-T cells may be generated in higher numbers and immigrate more rapidly into the periphery.

In contrast to lymph nodes and spleen, we observed that the percentage of small intestinal γδTCR<sup>+</sup> IEL was consistently decreased in CCR9<sup>−/−</sup> mice. In the gastrointestinal tract, CCL25 expression is restricted to the small intestinal epithelium (Fig. 5A) (11, 13–15). Interestingly, although the percentage of γδTCR<sup>+</sup> IEL in the small intestine of CCR9<sup>−/−</sup> mice was decreased, we found that the percentage of γδTCR<sup>+</sup> IEL in the large intestine was increased (Fig. 5, B and C). The large intestinal γδTCR<sup>+</sup> IEL in CCR9<sup>−/−</sup> mice were phenotypically similar to those in CCR9<sup>+/+</sup> mice (i.e., they did not resemble small intestinal IEL; data not shown), indicating that the increase in large intestinal γδTCR<sup>+</sup> IEL is not due to migration of cells from the small intestine to the large intestine.

All IEL from human small intestine express CCR9 and respond to CCL25 (13–15), and in mice both γδTCR<sup>+</sup> and αβTCR<sup>+</sup> IEL have been shown to express CCR9 mRNA (21). Thus, CCR9 might be important for the recruitment of mature γδ-T cells to the intestinal mucosa. Indeed, the observation that large intestinal IEL were not decreased in CCR9<sup>−/−</sup> mice localizes the defect to the site of CCL25 production (Fig. 5, A and B). Consistent with this idea is the finding that small intestinal αβTCR<sup>+</sup>CD8αβ<sup>+</sup> IEL, which are thought to be thymically derived and would therefore migrate from the periphery to the small intestine, are also reduced in CCR9<sup>−/−</sup> mice (Fig. 5F). Another possibility is that in the absence of CCR9, γδTCR<sup>+</sup> IEL fail to be retained in the small intestine. However, the preferential loss of TCR-VγδI/H<sup>+</sup> IEL and our inability to find these cells in lymph node or spleen (Fig. 5E...
and data not shown) suggest that there may be a direct role for CCR9 in Vγ5/δ64+ IEL development or that these cells may fail to survive if they are unable to home to the proper site. The accumulation of diverse subsets of γδ-T cells in the periphery of CCR9−/− mice could also reflect the pool of cells that initially migrate to the intestine and may differ significantly from the population that ultimately becomes established as IEL. Finally, the increase in peripheral γδ-T cells may reflect a shift in a dynamic equilibrium between the small intestine and the peripheral pool. The lower numbers of γδTCR+ IEL in CCR9−/− mice even in the face of what may be a compensatory increase in these cells in the periphery suggests an important role for CCR9 in establishing this equilibrium.

CCR9 and CCL25 mRNA are also detected in the small intestine of Rag-1−/− mice, which lack mature lymphocytes (21), raising the possibility that CCR9 plays a role in early mucosal T cell development and/or recruitment of IEL precursors to the small intestine. Cryptopatches are multiple clusters of c-kit+ IL-7Rα+ Thy1+ lymphocytes located in the crypt lamina propria of the murine intestine (45). CCR9 may be involved in cryptpatch formation and/or extrathymic γδTCR+ IEL development, perhaps by regulating the migration of progenitor cells from the fetal liver, fetal thymus, or adult bone marrow to the small intestine. γδTCR+ IEL are greatly reduced in nude mice and neonatal thymectomized mice (46). Transplantation of fetal or neonatal thymus, but not IEL, into nude mice results in the generation of a substantial number of thymically derived adult thymus, into nude mice results in the generation of a substantial number of thymically derived IEL (46, 47).

IEL development or that these cells may fail to migrate or to undergo a shift in a dynamic equilibrium between the small intestine and the peripheral pool. The lower numbers of γδTCR+ IEL in CCR9−/− mice even in the face of what may be a compensatory increase in these cells in the periphery suggests an important role for CCR9 in establishing this equilibrium.

In summary, these results identify a role for CCR9 in αβ- and γδ-T cell development that may include regulating the migration of progenitor cells to specific sites of T lymphopoiesis. The selective expression of CCL25 in the small intestine and the deficiency of small, but not large, intestinal γδ-T cells also suggest the existence of distinctive mechanisms of lymphocyte recruitment that may permit functional specialization of immune responses in different segments of the gastrointestinal tract.

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References


