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Ectopic Expression of the Murine Chemokines CCL21a and CCL21b Induces the Formation of Lymph Node-Like Structures in Pancreas, But Not Skin, of Transgenic Mice

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The CC chemokine CCL21 is a potent chemoattractant for lymphocytes and dendritic cells in vitro. In the murine genome there are multiple copies of CCL21 encoding two CCL21 proteins that differ from each other by one amino acid at position 65 (either a serine or leucine residue). In this report, we examine the expression pattern and biological activities of both forms of CCL21. We found that although both serine and leucine forms are expressed in most tissues examined, the former was the predominant form in lymphoid organs while the latter was predominantly expressed in nonlymphoid organs. When expressed in transgenic pancreas, both forms of CCL21 were capable of inducing the formation of lymph node-like structures composed primarily of T and B cells and a few dendritic cells. Induction of lymph node-like structures by these CCL21 proteins, however, could not be reproduced in every tissue. For instance, no lymphocyte recruitment or accumulation was observed when CCL21 was overexpressed in the skin. We conclude that both forms of CCL21 protein are biologically equivalent in promoting lymphocyte recruitment to the pancreas, and that their ability to induce the formation of lymph node-like structures is dependent on the tissues in which they are expressed. The Journal of Immunology, 2002, 168: 1001–1008.

Our group and others have identified multiple copies of CCL21 genes in the murine genome. These genes encode two proteins that differ by one amino acid (serine or leucine) at position 65 (23, 24). The CCL21a gene encodes the serine form, whereas both CCL21b and CCL21c genes encode the leucine form. The plt mice lack the expression of the CCL21a gene in lymphoid organs due to deletion of this gene (23, 24). However, CCL21 message (presumably encoded by CCL21b and/or CCL21c) can still be found in nonlymphoid tissues of plt mice, suggesting that the CCL21a gene is expressed in lymphoid organs and that CCL21b and -c genes are expressed in nonlymphoid tissues. However, inferences on the expression pattern of CCL21 genes based on the analysis of the plt mutant may be inaccurate, because the entire locus may be dysregulated. To clearly define the expression patterns of both the CCL21a and CCL21b genes, we have used quantitative PCR. We report here that both genes are expressed in multiple tissues, with CCL21a being the predominant form in the lymphoid organs and CCL21b the predominant form in nonlymphoid tissues. In addition, we show here that both serine and leucine forms of CCL21 are biologically equivalent. When expressed in the pancreas of transgenic mice both CCL21a and CCL21b drive recruitment of lymphocytes into the pancreas and formation of lymph node-like structures. This property seems to be tissue dependent, because transgenic expression of CCL21b in the skin or brain (51) is not associated with lymphocyte recruitment or lymphoid neogenesis.

Materials and Methods

Transgene construction and microinjection

RCC21a and RCC21b. BosXI fragments of CCL21 genomic DNA encoding CCL21a, 3.45 kb, or CCL21b, 3.55 kb, were isolated from two independent bacterial artificial chromosome clones (23) and subcloned into the EcoRV/SalI sites of the RIP-TNF-α-pBS plasmid containing rat insulin

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promoter II (RIP) (25). The TNF-α fragment in RIP-TNF-α-pBS was replaced with the CCL21 genomic DNA. Transgenes were released from the vector by restriction digest with NotI and ApaI restriction enzymes.

**KCCl21b.** A 1-kb DNA fragment containing all four exons and three introns of mouse CCL21b genomic DNA was amplified by PCR using specific rat CCL21 sequences as primers (26). The transgene (KCCl21b) was isolated from the plasmid by restriction digest with EcoRI and NotI.

Separation of the transgenes from vector DNA was accomplished by zonal sucrose gradient centrifugation as previously described (27). Fractions containing the transgene were pooled, microcentrifuged through Microcon-100 filters (Amicon, Beverly, MA), and washed five times with microinjection buffer (5 mM Tris-HCl (pH 7.4), 5 mM NaCl, and 0.1 mM zonal sucrose gradient centrifugation as previously described (27). Fractions containing the transgene were pooled, microcentrifuged through Microcon-100 filters (Amicon, Beverly, MA), and washed five times with microinjection buffer (5 mM Tris-HCl (pH 7.4), 5 mM NaCl, and 0.1 mM EDTA).

### Generation of transgenic mice

Transgenes were resuspended in microinjection buffer (5 mM Tris-HCl (pH 7.4), 5 mM NaCl, and 0.1 mM EDTA) to a final concentration of 1–5 ng/μl microinjected into (C57BL/6 × DBA/2)F1. The Jackson Laboratory, Bar Harbor, ME, eggs, and transferred into oviducts of ICR (Charles River Laboratories, Wilmington, MA) foster mothers, according to published procedures (28). At 10 days after birth, a piece of tail from the resulting founder was clipped for DNA analysis. Identification of transgenic founders was conducted by PCR analysis, as previously described (29). Identification of the transgenic mice was accomplished by PCR amplification of mouse tail DNA using specific primer sets. Specifically, the primers used for each transgene are listed as follows: KCCl21b: forward, 5'-cggagcagctctctcagc-3'; reverse, 5'-gctctcctctctcagcagc-3'; and KCCl21a: forward, 5'-taaggacctagctctc-3'; and reverse, 5'-agggacaagccctgag-3'. The endogenous ZP3 gene, used as an internal control, was amplified with the following primers: forward, 5'-cagctctctacatcacctgcca-3' and reverse, 5'-cactgggaagagacactcag-3'. The resulting transgenic animals were kept under specific-pathogen-free conditions. All animal experiments were performed following the guidelines of the Schering-Plough animal care and use committee.

**Histological analysis**

Tissues for light microscopic examination were fixed by immersion in 10% phosphate-buffered Formalin and then processed for paraffin sections. Routinely, 5-μm sections were cut and stained with H&E. For immunohistochemistry, formalin-fixed sections were transferred onto fresh medium, and cells that had migrated into the medium during the first 48 h were counted in a hemocytometer, in which dendritic cells, dead cells, and other viable cells were morphologically distinguishable. Dendritic cells that migrated between 48 and 96 h after culture were counted and identified by fluorescence microscopy with directly conjugated primary Abs in FACS buffer for 20 min at 4°C. The dark mAbs to the following mouse surface markers were purchased from BD Biosciences Inc. (San Jose, CA): anti-CD4 (clone RM4-5), anti-CD11c (clone N418), anti-pan NK (clone PK136), anti-B220 (clone RA3-6B2). Events were acquired on a BD Biosciences FACScan and analyzed using CellQuest software.

### Flow cytometry and cell preparation from ears

To prepare single-cell suspension, the ears were first separated into dorsal and ventral halves. The dorsal half was placed fur side up in 8 ml of 0.25% trypsin and EDTA (Life Technologies) for 30 min at 37°C. The preparation was then diluted 1/4 with 1× PBS. The epidermis and dermis were transferred with forceps to fresh 1× PBS and then drawn up and down ~20 times with a 10-ml syringe. Following the addition of 8 ml of 10% FBS in 1× PBS, the cell suspension was transferred onto a 100-μm pore size nylon mesh (Falcon; BD Biosciences, Franklin Lakes, NJ). The cells were pelleted at 1000 rpm for 10 min and resuspended at 1× 10^6 cells/ml in 1× PBS, 5% FBS, and 0.02% sodium azide (FACS buffer).

Cells (10^5–10^6) were incubated with 5 μg/ml Fc block (BD PharMingen) and 300 μg/ml mouse IgG (Pierce, Rockford, IL). Cells were stained with directly conjugated primary mAbs in FACS buffer for 20 min at 4°C in the dark. mAbs to the following mouse surface markers were purchased from BD PharMingen: B220 (RA3-6B2), CD11c (HL-3), MHC II (25-9-17), and TCRR5 (H57-597). To determine viability, samples were subse- quently stained with 20 μl of 5 μg/ml propidium iodide (Calbiochem, La Jolla, CA). Events were acquired on a BD Biosciences FACScan and analyzed using CellQuest program.

### Migration of Langerhans cells (LC)

The experiment was performed according to a published method (32). In brief, ear skin from transgenic and control mice was separated from the cartilage, and the ventral sides were floated on 1.5 ml of culture medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 0.05 mM 2-ME, and 50 μg/ml gentamicin) in 24-well plates. After 48 h, the explants were transferred onto fresh medium, and cells that had migrated into the medium during the first 48 h were counted in a hemocytometer, in which dendritic cells, dead cells, and other viable cells were morphologically distinguishable. Dendritic cells that migrated during the first 48 h are therefore typically hairy or veiled. Dendritic cells that migrated between 48 and 96 h after culture were counted and identified in the same way.

To determine the numbers of LC in epidermal and dermal sheets, FITC-labeled anti-I-A* (clone 2G9; BD PharMingen) was used in immunofluorescent staining. In brief, epidermal and dermal sheets were prepared before the onset and at the end of culture. For this purpose ear skin was separated from the cartilage, and the ventral sides were floated on 1.5 ml of culture medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 0.05 mM 2-ME, and 50 μg/ml gentamicin) in 24-well plates. After 48 h, the explants were transferred onto fresh medium, and cells that had migrated into the medium during the first 48 h were counted in a hemocytometer, in which dendritic cells, dead cells, and other viable cells were morphologically distinguishable. Dendritic cells that migrated during the first 48 h are therefore typically hairy or veiled. Dendritic cells that migrated between 48 and 96 h after culture were counted and identified in the same way.

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with a calibrated ocular grid (20–40 fields/sample; n = 3) at ×40 magnification. Statistical analysis was performed using the Mann-Whitney U test.

**Results**

**CCL21a and CCL21b genes are expressed in multiple tissues**

To examine the expression pattern of the different CCL21 genes, we used quantitative PCR analysis. Because these genes are highly homologous, we first tested the specificity of the primers on two different plasmid DNAs encoding the CCL21a and the CCL21b genes, respectively. As shown by the lower Ct values of CCL21a plasmid compared with those of CCL21b plasmid in Fig. 1A, CCL21a-specific primers preferentially amplified the CCL21a plasmid. For example, if 1 ng each of CCL21a and CCL21b template plasmids were amplified together, the CCL21a-specific primers would have at least 10,000-fold higher amplification efficiency for the CCL21a than CCL21b plasmid. Similarly, the CCL21b-specific primers showed a strong preference toward the CCL21b plasmid (Fig. 1B). Additional evidence for the specificity of the CCL21 primers was derived from analysis of RNAs extracted from plt lymphoid tissues. No amplification was detected in any plt tissues with CCL21a-specific primers (not shown), consistent with deletion of this gene in the plt mutant (23).

To investigate the expression pattern of CCL21a and CCL21b, we extracted RNA from thymus, mesenteric lymph node, spleen, liver, small and large intestines, stomach, heart, lung, kidney, muscle, and testis from B6D2 mice; reverse transcribed the RNA; and performed quantitative PCR analysis. We found CCL21a to be expressed at high levels in lymphoid tissues such as thymus, mesenteric lymph node, and spleen and at low levels in most nonlymphoid tissues analyzed (Fig. 1C). On the other hand, CCL21b was expressed in all tissues examined (Fig. 1D); the highest levels were found in lung and thymus. As expected, no CCL21a expression was detected in any of the plt tissues examined, while very low levels of CCL21b (11–23.5 fg/25 ng cDNA) were detected in lymphoid tissues of plt mice (data not shown).

**Generation of transgenic mice expressing CCL21a or CCL21b in the pancreatic islets**

To study the biological function of the CCL21a and CCL21b in vivo, we generated transgenic mice constitutively expressing either gene in pancreatic islets. To this end we used a promoter, RIP (Fig. 2A) (25). These transgenic mice are referred to as RCCL21a and RCCL21b, respectively. A total of 7 and 10 founders was generated for RCCL21a and RCCL21b, respectively. Five independent lines of mice were derived for each transgene. All transgenic mice carrying either form of CCL21 developed normally and were fertile.

Transgene expression in the islets was analyzed by immunohistochemical staining using an anti-CCL21 Ab that does not distinguish between the two forms of CCL21 protein. As shown in Fig. 2B, high levels of CCL21 protein were expressed in the endocrine pancreas of both RCCL21a and RCCL21b transgenic, but not control, mice. Next, we examined the expression of the transgenes in kidney because this is an organ occasionally targeted by the RIP promoter (33). We did not detect transgene expression in the kidneys of any of the RCCL21 lines analyzed (n = 3; data not shown).

Both CCL21a and CCL21b proteins promote recruitment of lymphocytes and dendritic cells into pancreatic islets

CCL21 has been shown to induce migration of lymphocytes and dendritic cells in vitro (3, 5, 6, 12). To determine whether CCL21 could induce lymphocyte recruitment in vivo, we examined H&E-stained paraffin sections of transgenic pancreas microscopically. Mononuclear infiltrates of varying sizes were found in the pancreatic islets of both RCCL21a and RCCL21b transgenic mice (Fig.

**FIGURE 1.** Quantitative PCR analysis with TaqMan. A and B, Ct for CCL21a (●) and CCL21b (■) plasmids with either CCL21a-specific (A) or CCL21b-specific (B) primers was shown at plasmid DNA concentrations from 1 ng to 1 fg. Ct values correspond to the cycle number at which the amplification plot crosses the defined fluorescence threshold. RNAs isolated from lymphoid and nonlymphoid tissues of B6D2 mice were determined using CCL21a-specific (C) or CCL21b-specific (D) primers in PCR using a TaqMan instrument. Th, thymus; ML, mesenteric lymph node; Sp, spleen; Li, liver; SI, small intestine; LI, large intestine; St, stomach; He, heart; Lu, lung; Ki, kidney; Mu, muscle; Te, testis.
The large cellular infiltrates observed in the islets of both RCCL21a and RCCL21b resembled those observed in insulitis. Despite this similarity, none of the RCCL21a and RCCL21b mice examined (n = 26) developed diabetes within the first year of life.

To further characterize these cellular infiltrates, we applied immunohistochemical staining using Abs against several leukocyte surface makers. Both T and B lymphocytes were present in the cellular infiltrates and appeared to be organized into separate compartments with T cells at the center of the infiltrates (Fig. 3C). CD8 T cells (Fig. 3D), CD11c+ cells (Fig. 3E), and very small numbers of F4/80+ cells (data not shown) were also found among the cellular infiltrates. No NK+ cells were observed using the DX5 Ab that reacts with the majority of NK cells (data not shown). Again, similar immunohistochemistry results were observed for both RCCL21a and RCCL21b transgenic mice.

**Lymph node-like structures were found in pancreatic islets of both RCCL21a and RCCL21b transgenic mice**

As demonstrated by the previous results the cellular composition of the pancreatic infiltrates was similar to that seen in secondary lymphoid organs. To further define the nature of these aggregates we examined a number of other parameters. We started by examining the expression of adhesion molecules such as MadCAM-1 and PNAd, adhesion molecules that are specifically expressed in secondary lymphoid structures. The expression of these two molecules was found in the vessels present in the cellular infiltrates of both RCCL21a and RCCL21b mice, but not in the islets of wild-type controls (Fig. 3, F and G). Next we examined whether stromal reticulum could be detected within the lymphoid aggregates. The presence of stromal reticulum, as defined by the presence of ER-TR7-positive cells, was detected in the pancreas of transgenic animals expressing both CCL21a and CCL21b (Fig. 3H). No ER-TR7-positive cells were found in the islets of wild-type controls. These results indicate that expression of CCL21a or CCL21b in the pancreas is sufficient to induce the formation of lymph node-like structures.

**Generation of transgenic mice expressing CCL21b in the skin**

To further investigate the role of CCL21 in lymphocyte recruitment in vivo, we generated transgenic mice expressing CCL21b in keratinocytes. To this end, we used the human K14 promoter that targets expression of transgenes to the basal cell layer of the epidermis (Fig. 4A). A total of 15 transgenic founders were generated by microinjection, as determined by PCR genotyping. These transgenic mice are referred to as KCCL21b mice.

Endogenous CCL21 expression in the lymphatics was observed in both wild-type and transgenic mouse skin by immunohistochemistry (Fig. 4B). However, expression of CCL21 in the epidermal cell layer and the hair follicles was only found in transgenic, but not wild-type control, mice. Two independent lines (lines 32 and 72) with different levels of transgene expression (Fig. 4B) were propagated for further analysis. No gross phenotypic abnormalities were observed in the KCCL21b transgenic mice during development or in adulthood.

**CCL21b does not promote recruitment of lymphocytes and dendritic cells to the skin**

To investigate whether the K14-driven expression of CCL21b led to leukocyte recruitment, we collected samples from dorsal skin, ear, esophagus, stomach, vagina, and tongue and examined them by light microscopy. No significant differences were observed between transgenic and wild-type control tissues (Fig. 5, top panel). Tissue sections from both wild-type control and KCCL21b transgenic mice were further analyzed by immunohistochemistry, using anti-CD3ε, anti-CD4, and anti-TCRβ Abs. As shown in Fig. 5, the number of TCRβ-positive cells was comparable to that found in the control. Similar results were obtained with either anti-CD4 or anti-CD3ε Abs (data not shown). Flow cytometry was used next to study whether expression of CCL21b was associated with changes in the number of TCR+ cells. Flow cytometric analysis did not reveal changes in the number of TCRβ+, B220+, or CD11c+ cells in the transgenic ears compared with the control ears (Fig. 6). These results indicated that expression of CCL21 is not sufficient to promote lymphocyte or dendritic cell recruitment to the skin.

**Ectopic expression of CCL21b in the skin is associated with reduced migration of LC**

CCL21 is normally expressed by the lymphatic endothelium in the skin (Fig. 4B), but it is unclear what physiological role it has in this
tissue. Studies with anti-CCL21 Abs have suggested that CCL21 may be involved in mediating the migration of skin-derived dendritic cells into the draining lymph nodes (11). To determine whether expression of CCL21b by keratinocytes would alter the migration of dendritic cells from the skin, we tested the migration of LC in ear organ cultures. Before culture, a small piece of the epidermis was stained with an anti-MHC class II Ab to assess the number of LC in the ear explants. In agreement with the results from the flow cytometric analysis reported above, similar numbers

**FIGURE 3.** Characterization of the cellular infiltrates in islets of CCL21 transgenic mice. H&E-stained paraffin sections of RCCL21a (A) and RCCL21b (B) pancreas showing cellular infiltrates in their islets. Cell infiltrates in the pancreatic islets of RCCL21 mice were analyzed by immunohistostaining with FITC-labeled anti-CD4 (green) and PE-labeled anti-B20 (red; C), anti-CD8 (D), and anti-CD11c (E) Abs. Expression of adhesion molecules in leukocyte infiltrates of RCCL21 islets was observed using anti-MAdCAM-1 (F) and anti-PNAd (G). H, The induction of ER-TR7, a marker for stromal reticulum structure, was detected with an anti-ER-TR7 Ab. Original magnification: A and B, ×100; F, ×200; and C–E, G, and H, ×400.

**FIGURE 4.** Generation of KCCL21b transgenic mice. A, KCCL21b transgene. B, Expression of CCL21 in the skin of KCCL21b transgenic mice. Skin sections from animals in KCCL21b lines 32 and 72 are shown.

**FIGURE 5.** Expression of CCL21b in the skin does not cause lymphocyte infiltration. Top panel, H&E-stained paraffin sections from wild-type (WT) and both lines of KCCL21b mice. Bottom panel, TCRβ immunostaining of skin from wild-type and both lines of KCCL21b mice. Arrows indicate the few scattered dermal T cells.
of MHC II-positive LCs were found in wild-type and KCCL21b ears (1260 ± 130 LC/mm² in wild-type (Fig. 7A) vs 1240 ± 70 LC/mm² in KCCL21b (Fig. 7B)). After 96 h of organ culture, the LC that remained in the epidermis were counted. In wild-type ears, there was a dramatic decrease in LC densities (90 ± 70 LC/mm²; Fig. 7C), whereas the numbers in KCCL21b ears were much less reduced (850 ± 380 LC/mm²; Fig. 7D). This difference was highly significant (p < 0.001; Fig. 7E). Notably the LC of the KCCL21b mice were in an activated state and ready to leave the epidermis, as suggested by their strong MHC class II expression and their rounded shape (Fig. 7D).

To complement this picture, the cells that migrated from the ear explants into the culture media were counted at 48 and 96 h in organ culture. There was a significant reduction in the number of dendritic cells that migrated out of the transgenic ear explants both in the first 48 h of culture and between 48 and 96 h of culture (Fig. 7F). These results show that expression of CCL21b in the keratinocytes inhibited migration of LC from the skin.

Discussion

In this report, we demonstrate that both CCL21a and CCL21b genes are expressed in multiple murine tissues (thymus, spleen, mesenteric lymph node, liver, small and large intestines, stomach, heart, lung, kidney, muscle, and testis), and that they are biologically equivalent in promoting lymphocyte recruitment to the pancreas. We also demonstrate that overexpression of CCL21b in the skin does not alter the number of lymphocytes and dendritic cells, but reduces the migration of dendritic cells from this tissue.

Because CCL21 is expressed normally in several tissues and has been shown to chemoattract lymphocytes and dendritic cells in vitro, it has been suggested that it may be an important regulator of their trafficking in vivo. The discovery of at least three murine
CCL21 genes (23, 24) encoding two different proteins prompted a series of questions, among them, what are the patterns of expression of these genes and what are their biological functions. Before this study it was known that expression of CCL21 could be detected by Northern blot analysis in multiple murine tissues (3, 23). Furthermore, it was known that CCL21 expression could be detected in nonlymphoid tissues of plt mice, which had a deletion of the CCL21a gene, suggesting the expression of either CCL21b or CCL21c (which are identical in their coding region) in these tissues (23, 24). However, it was unclear whether the analysis of CCL21 expression in plt mice would reflect the physiological regulation of the locus. Formally it could be argued that this pattern of expression could have resulted from an abnormal regulation of the mutated locus. Another unresolved question was whether the CCL21a gene would be expressed in lymphoid as well as nonlymphoid tissues. Using primers specific for the two forms of the CCL21 protein (CCL21a and CCL21b/c), we demonstrate here that CCL21a is expressed in most tissues examined and that its highest levels of expression are found in lymphoid structures. The levels of expression of CCL21a in the nonlymphoid tissues are low. In contrast, the CCL21b gene is expressed in all nonlymphoid organs (especially the lung) and at very low levels in spleen and mesenteric lymph nodes. Taken together, these results indicate that the closely linked CCL21 genes are differentially regulated at the transcriptional level.

Leukocyte trafficking from blood vessels into tissue parenchyma is a multistep process (34, 35). Genetic evidence supporting a critical role for chemokines in this process has accumulated in the recent years (36). For instance, tissue-specific expression of the murine CXC chemokine KC in thymus, skin (26), brain (37), lung (38), or heart (S. A. Lira, unpublished observations) induces neutrophil recruitment into tissues. Similarly, the expression of the murine CC chemokine JE in thymus, brain (39), pancreas (40), lung (41), or heart (42) is associated with macrophage recruitment. Recently, two studies have demonstrated tissue-specific accumulation of lymphocytes upon expression of chemokines in transgenic mice. Luther et al. (43) have demonstrated accumulation of B and T cells in mice expressing BLC (CXCL13) in the pancreas. In addition, Fan et al. (44) reported the generation and analysis of animals expressing CCL21 in pancreas. Similar to what has been reported by Fan et al., we observed that expression of CCL21 in pancreas induces the recruitment and stereotypical arrangement of lymphocytes in the pancreatic islets, with T cells being surrounded by B cells, the presence of low number of dendritic cells, and the expression of adhesion molecules. Here we extend these observations by showing that both forms of CCL21 (Leu or Ser) can induce the formation of lymph node-like structures in the pancreas. Furthermore, we suggest a tissue-specific requirement for the biological activity of these molecules in the pancreas, because the expression of CCL21 in skin or brain (51) does not induce the same phenomenon.

What could account for the tissue-specific ability of CCL21 proteins to induce the formation of lymph node-like structures? One hypothesis would be that CCL21 induces a permissive "endothelial" environment for initial recruitment of lymphocytes and subsequent development of these structures. CCL21 could directly or indirectly induce the expression of the requisite adhesion molecules (such as MadCAM-1 and PNAd), which could then facilitate initial infiltration of lymphocytes. At least one of the CCL21 receptors, CXCR3, has been identified in vascular structures (45, 46) and could in theory mediate this effect. Alternatively, CCL21 could act on a bystander cell to induce the production of factors that would affect the expression of adhesion molecules in the endothelium. Such a bystander cell could be located within the pancreatic islet or be one of the initially recruited leukocytes. In transgenic mice expressing BLC in the pancreas, induction of the adhesion molecules is dependent on the presence of lymphotoxin $\alpha_1\beta_2$ (43). It is unknown at this point whether CCL21 induces lymphotoxin $\alpha_1\beta_2$, and to what extent it may contribute to the findings reported here. Regardless of the site of CCL21 action (endothelium vs bystander cell), one would have to propose that the activation of these mechanisms would take place preferentially in the pancreas, because no induction of adhesion molecules has been found in skin or brain upon overexpression of CCL21 (our unpublished observations). These differences could be due to the different vascular beds serving these tissues. The pancreatic islets are highly vascularized, and the functional capacity of this endothelium may differ from that of other vascular beds (47). Finally, it is theoretically possible that lymphocytes may be recruited by an as yet unidentified mechanism driven by CCL21 presented in the surface of the endothelium. In this case the differences in the recruiting properties of CCL21 would depend on the ability of the endothelium of the different tissues to present it to the circulating lymphocytes.

Mice lacking CCR7 and plt mice (which lack both CCL21 and CCL19 expression in lymphoid organs) have reduced numbers of dendritic cells in lymph nodes. Mature dendritic cells express CCR7 (11, 48, 49), which could mediate their migration toward the lymphatics, a site of abundant expression of CCL21. Thus, it would be expected that ectopic (basal cell layer of the epidermis) expression of CCL21 would dysregulate dendritic cell numbers or migration. In this study, we observed that epidermal CCL21 overexpression does not change the number of dendritic cells in the skin, but it significantly reduced the mobility of dendritic cells from this tissue. The overexpression of CCL21 in the epidermis may have disrupted a chemokine gradient generated by expression of CCL21 in dermal lymphatics. This chemokine gradient may be essential for normal migration of LC and dermal dendritic cells, particularly into the dermal lymphatics. Evidence for this has recently been corroborated in skin explant culture models (11, 16, 50). Alternatively, the ectopic expression of CCL21 in the epidermis may have disrupted the ability of these cells to migrate by desensitizing CCR7 (9, 21). Epidermal CCL21 overexpression does not affect the number of dendritic cells in the skin in the steady state, but may be important during infection or inflammation. Experiments to further address the mechanistic basis of these findings are currently underway.

In conclusion, we have observed that the ability of CCL21 to induce lymphocyte recruitment is context dependent. This property is in clear contrast to that shown by neutrophil and macrophage chemotactrant chemokines such as KC and JE, which are sufficient to promote recruitment of target cells to multiple tissues. These findings highlight further complexity in the chemokine system and suggest that the constitutively expressed homeostatic chemokines (such as CCL19 and CCL21) have specific requirements for their biological activity. Understanding the nature of these requirements will certainly lead to a better understanding of the lymphoid system and contribute to the development of CCL21-based therapies.

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