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Central Nervous System Inflammation and Neurological Disease in Transgenic Mice Expressing the CC Chemokine CCL21 in Oligodendrocytes

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To study the biological role of the chemokine ligands CCL19 and CCL21, we generated transgenic mice expressing either gene in oligodendrocytes of the CNS. While all transgenic mice expressing CCL19 in the CNS developed normally, most (18 of 26) of the CCL21 founder mice developed a neurological disease that was characterized by loss of landing reflex, tremor, and ataxia. These neurological signs were observed as early as postnatal day 9 and were associated with weight loss and death during the first 4 wk of life. Microscopic examination of the brain and spinal cord of CCL21 transgenic mice revealed scattered leukocytic infiltrates that consisted primarily of neutrophils and eosinophils. Additional findings included hypomyelination, spongiform myelopathy with evidence of myelin breakdown, and reactive gliosis. Thus, ectopic expression of the CC chemokine CCL21, but not CCL19, induced a significant inflammatory response in the CNS. However, neither chemokine was sufficient to recruit lymphocytes into the CNS. These observations are in striking contrast to the reported activities of these molecules in vitro and may indicate specific requirements for their biological activity in vivo. The Journal of Immunology, 2002, 168: 1009–1017.

Lymphocytes normally enter and survey the CNS constantly in small numbers (1, 2). Their presence in the CNS increases in various diseases, such as multiple sclerosis (3, 4) and viral infections (5), but the mechanisms involved in their trafficking under both physiological and pathological conditions are poorly understood. Candidate molecules mediating lymphocyte trafficking have been recently identified (6, 7). Among these molecules are small, secreted cytokines with chemotactic function known as chemokines (for review see Refs. 8–10). Evidence supporting a role for chemokines in mediating leukocyte recruitment into the CNS is derived from studies in transgenic mice which demonstrate that influx of neutrophils (11) and macrophages (12) occurs following CNS-specific expression of the chemokines KC and JE. Other studies have documented expression of chemokines in autoimmune and posttraumatic brain models, which are associated with leukocyte infiltration (13–16). Leukocyte recruitment into the CNS has also been demonstrated by intracerebroventricular injection of the chemokine C10 (16). These in vivo models clearly demonstrate that the CNS is permeable to leukocytes, particularly neutrophils and macrophages, when the appropriate chemokines are expressed. Recently, the chemokines CCL19 and CCL21 have been implicated in the migration of lymphocytes in vitro and in vivo. In this report we study the ability of these chemokines to mediate the influx of lymphocytes into the CNS.

CCL21, also known as 6Ckine (17), secondary lymphoid chemokine (18), Exodus-2 (19), and thymus-derived chemotactic agent-4 (20), is a CC chemokine whose amino acid sequence exhibits an unusual pattern of six conserved cysteines, two of which are located in an unique, highly charged, carboxyl-terminal extension (17–20). CCL21 is constitutively expressed at high levels in secondary lymphoid organs, particularly lymph nodes and spleen in both human and mouse (17–20). Expression of CCL21 has also been localized to the high endothelium venules of lymph nodes and Peyer’s patches (21, 22). Recombinant murine CCL21 is chemotactic in vitro for thyocytes, naïve T cells, mature dendritic cells, and, at high concentrations, naïve B cells, but not for macrophages or neutrophils (17, 18, 20, 21, 23, 24). CCL21 binds and induces calcium flux in cells transfected with CCR7 (25). Binding to CXCR3-transfected cells has also been reported for murine CCL21 (26) but not for human CCL21 (27). Interaction of CCL21 with receptors on lymphocytes is thought to account for the rapid integrin-dependent arrest of lymphocytes rolling under physiological shear (28, 29).

CCL19, also known as macrophage-inflammatory protein (MIP)3B (30) or EBV-induced gene-1 ligand chemokine (31), is another ligand for CCR7. It is expressed in thymus and secondary lymphoid organs (30, 31). CCL19, similar to CCL21, attracts thyocytes, T and B lymphocytes, and mature dendritic cells (24, 32, 33). Both CCL19 and CCL21 are reportedly expressed by stromal cells in the T cell zone of lymph nodes (34).

In the mouse genome, CCL19 and CCL21 are encoded by multiple genes. CCL21 is represented by at least two genes encoding for two different forms of CCL21 protein (CCL21a and CCL21b) which differ by one amino acid at position 65 (35, 36). Mutations in CCL19 and CCL21 genes are associated with specific defects in

Abbreviations used in this paper: MIP, macrophage-inflammatory protein; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; P, postnatal day; BBB, blood-brain barrier.
lymphocyte trafficking. The mouse strain, paucity of lymph node T cells (plt), which lacks CCL21a and the only functional copy of CCL19 (34–36), has deficits in the migration of both T lymphocytes and dendritic cells into lymph nodes (37). In addition, mice lacking CCR7, a receptor for both CCL19 and CCL21, have impaired migration of lymphocytes into secondary lymphoid organs and develop a phenotype that is similar to that presented by the plt mice (38).

To determine whether CCL19 or CCL21 could alter lymphocyte influx/trafficking into the CNS, we generated transgenic mice expressing either of these molecules in oligodendrocytes.

Materials and Methods

Transgene construction and microinjection

Transgenes were generated by cloning the coding region of either a 441-bp murine CCL21b (17) or a 484-bp murine CCL19 (30) cDNA into the Xhol-Sall site of the plasmid pMBP, generously provided by Dr. R.A. Lazza-ri (Mount Sinai Medical Center, New York, NY). This plasmid contains 1.9 kb (−1907 to +36 bp) of the promoter/enhancer of the myelin basic protein (MBP) gene. Additional genomic sequences include splice and polyadenylation signals supplied by exon 6, intron 6, and exon 7 of the human proteolipid (39). The resulting transgenes are referred to as MBPCCL21 and MBPCCL19, respectively. Both transgenes were isolated from the plasmids by restriction digest with NotI. Separation of the transgenes from vector sequences was accomplished by zonal sucrose gradient centrifugation as described (40). 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, 0.1 mM EDTA).

Generation of transgenic mice

Each transgene was resuspended in microinjection buffer (5 mM Tris-HCl (pH 7.4), 5 mM NaCl, 0.1 mM EDTA) to a final concentration of 1–5 ng/μl, microinjected into (C57BL/6 × DBA/2F2) (The Jackson Labora-tory, Bar Harbor, ME) eggs, and transferred into oviducts of ICR (Charles River Breeding Laboratories, Wilmington, MA) foster mothers, according to published procedures (41). At 10 days after birth, a piece of tail from the resulting animals was clipped for DNA analysis. Identification of trans-genic founders was conducted by PCR analysis, as previously described (42). The following primer pairs were used to identify the transgenic mice by amplification of mouse tail DNA: for MBPCCL21, 5′-gcctgagaca ccaaggaac-3′ and 5′-tgacccttgagccctttcct-3′; for MBPCCL19, 5′-ggc gaagactgctgctgctgtg-3′ and 5′-gcctgagactgctgctgctgtg-3′. The endogenous ZP3 gene (5′-cagctcatacactcctca-3′ and 5′-cactggagagaacacgctca-3′) was used as an internal control for the amplification reactions. These primers amplify a 443-bp segment of the MBPCCL21 transgene, a 380-bp segment of the MBPCCL19 transgene, and a 511-bp segment of the ZP3 gene. PCR conditions were 95°C for 30 s, 60°C for 30 s, and 72°C for 60 s for 30 cycles. 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.

RNA analysis

RNA was extracted from tissues using Ultraspec RNA (Biotecx Laborato ries, Houston, TX) following specifications from the manufacturer. Total RNA (20 μg) was denatured, separated by gel electrophoresis, and blotted onto GeneScreen membrane (NEN, Boston, MA). A 441-bp fragment of the murine CCL21 cDNA and a 484-bp fragment of the murine CCL19 cDNA were radiolabeled using a random-priming DNA-labeling kit (Stratagene, La Jolla, CA) and used as probes in the hybridization of Northern blots.

Histological analysis

After euthanasia, tissues were either fresh-frozen with freezing medium for cryosection or fixed by immersion in 10% phosphate-buffered formalin and then processed for paraffin sections. Tissues for light microscopic examination were routinely processed, sectioned at 5 μm, and stained with H&E. For immunohistochemistry, fresh-frozen sections were first fixed with ice-cold acetone for 10 min and air-dried. Paraffin sections were deparaffinized and rehydrated to 1 × PBS before use. The sections were then incubated sequentially with biotin solution (Vector Laboratories, Burlingame, CA), avidin solution (Vector Laboratories), and 10% normal goat or rabbit se-
In contrast, 18 of 26 MBPCCL21 founders (or their progeny) developed a distinct neurological phenotype, characterized by loss of the righting reflex, tonic tremor during standing or walking, and ataxia. Most of the MBPCCL21 founders or offspring that developed neurological signs had reduced body weight and were either found dead within the first 4 wk of life or were infertile. Macroscopic and microscopic examination of non-neural tissues revealed no obvious cause of death except for wasting.

To investigate whether the neurological signs were associated with expression of the MBPCCL21 transgene, we examined the transgene expression in the brain of the MBPCCL21 animals by Northern blot analysis. Transgene expression was detected in the affected founders and correlated with the intensity of the signs. Animals showing high expression of the transgene showed more severe clinical abnormalities, whereas those showing low levels of expression demonstrated a nearly normal righting reflex and were generally more active than those expressing higher levels of the transgene. Founders that had no transgene expression did not show neurological signs (data not shown).

Due to poor health and premature death it was difficult to generate lines from most of the MBPCCL21 founders. However, transgenic offspring were derived from four founders that showed little or no disease (nos. 171, 296, 299, and 322). Interestingly, animals derived from founders 299 and 322 were mildly affected clinically and expressed very low levels of the transgene (Fig. 1C).

Founders 171 and 296 had mild disease but were most likely mosaic, because their progeny were highly affected and expressed high levels of the transgene. The studies reported in this work were mostly conducted with animals derived from founders 171 and 296. In progeny from both 171 and 296 founders, the first signs of neurological deficit were detected at postnatal day (P) 9. Similar to what was observed with most founders, these progeny also presented with severe neurological disease and died within the first month of life, preventing further expansion of the lines.

**FIGURE 1.** MBPCCL19 and MBPCCL21 transgenes and analysis of their expression in the CNS. *A*, Diagram of the MBPCCL21 and MBPCCL19 transgenes. The MBP promoter drives the expression of either murine CCL19 (upper panel) or CCL21b (lower panel) cDNA fused to the genomic DNA of the human proteolipid gene (hPLP). This genomic human proteolipid gene DNA provides introns, exons, and polyadenylation sequences. Translation of the hybrid mRNA terminates at the stop codon of the cDNAs. *B* and *C*, Northern hybridization. Brain RNAs isolated from wild-type, MBPCCL19 (*B*), or MBPCCL21 (*C*) mice were used to examine the transgene expression. cDNAs of CCL19 (*B*) or CCL21 (*C*) were used as probes in the Northern hybridization. The lane numbers indicate the transgenic line numbers.

**FIGURE 2.** Immunohistochemical analysis of CCL19 and CCL21 expression in the CNS. Shown are fresh-frozen (*A* and *B*) or paraffin (*C* and *D*) sections from wild-type (*A* and *C*), MBPCCL19 (*B*), and MBPCCL21 (*D*) brains stained with anti-CCL19 (*A* and *B*) or anti-CCL21 (*C* and *D*) Abs. No CCL19 or CCL21 staining was detected in wild-type control brains. cc, Corpus callosum; CA, pyramidal cell layer of the hippocampus. Bar = 80 μm.
Leukocyte infiltration in the CNS of MBPCCL21 mice

Routine histologic analysis of the H&E-stained paraffin sections of the medullar and overlying cerebellum (A) and spinal cord (B) from MBPCCL19 transgenic mice showed no leukocyte infiltration. Bar = 400 μm in A and 80 μm in B.

Ultrastructurally, significant changes were present in the cerebrum, cerebellum, medulla, and spinal cord. Perivascular infiltrates of neutrophils and eosinophils were present, as were individual neutrophils and eosinophils scattered throughout the parenchyma (Fig. 4D). A few macrophages were also seen adjacent to blood vessels and scattered throughout the parenchyma. Rare necrotic cells were also seen in the brain. Hypertrophic astrocytes containing bundles of intermediate filaments were sometimes observed. Astrocyte processes, especially adjacent to blood vessels, were also swollen.

To further investigate the cellular infiltrates in the CNS of both MBPCCL19 and MBPCCL21, immunohistochemical staining with several Abs was conducted. We were not able to detect B220-positive cells within the CNS parenchyma of either MBPCCL19 or MBPCCL21 transgenic mice (data not shown). Rare CD4- and CD3e-positive cells were detected in the parenchyma and periventricular areas in the brains of MBPCCL21 mice (Fig. 4E). Therefore, no significant infiltration of lymphocytes, either T or B

FIGURE 4. Neutrophil and eosinophil infiltration in the CNS of MBPCCL21 transgenic mice. A, A cluster of neutrophils and eosinophils in the brain. Bar = 15 μm. B, Neutrophils scattered throughout the neuropil of the medulla. Bar = 25 μm. C, Perivascular infiltrate of eosinophils in the medulla. Bar = 10 μm. D, Transmission electron micrograph of the brain of a MBPCCL21 mouse. There is a prominent perivascular eosinophilic infiltrate. Note the lobulated nuclei and numerous cytoplasmic granules. Bar = 1.7 μm. D, inset, Higher magnification of granules, showing elongated granules with a central core characteristic of eosinophils. E, Anti-CD4 immunohistochemical staining of a MBPCCL21 brain. No CD4-positive cells were detected in an area with significant inflammatory infiltrates. Bar = 93 μm. Samples were derived from 2- to 3-wk-old MBPCCL21 animals.
cells, was found in the CNS of MBPCCL19 and MBPCCL21 transgenic animals.

**Gliosis in the CNS of MBPCCL21 mice**

Inflammatory conditions of the CNS are often associated with changes in the activation of microglia. To determine whether there were changes in this cell population, we used an Ab against the activation marker F4/80 and studied the CNS of wild-type and MBPCCL21 mice between P8 and P18. In wild-type mice, a few lightly stained cells expressing F4/80 were detected throughout the brain and spinal cord parenchyma (Fig. 5A). In contrast, numerous F4/80-positive cells with morphological characteristics of ramified microglia/macrophages were observed in the spinal cord and brain of the MBPCCL21 mice. In the spinal cord, the F4/80-positive cells were first observed at P10 and the majority of these cells were located in the white matter. In animals at P11 and older, F4/80-positive cells were detected in both gray and white matter of the brain and spinal cord (Fig. 5B). Both the intensity of staining and the number of F4/80 positive cells increased with age. The expression of F4/80 closely mirrored the levels of transgene expression as determined by anti-CCL21 immunostaining and Northern analysis.

To determine whether astrocytes were activated in the MBPCCL21 mice, an anti-GFAP Ab was used for immunohistochemical staining. A marked increase in GFAP staining was found as early as P8 in the gray matter of the spinal cord. Increased numbers of GFAP-positive cells were observed in the medulla at the age of P10 and older. Like F4/80 staining, an age-related increase in the intensity of staining and numbers of GFAP-positive cells was also observed. At P17 GFAP staining could be seen in several areas of the CNS, particularly in the medulla and in deep cerebellar nuclei. As shown in Fig. 5, C and D, very intense GFAP staining was observed in both the gray and white matter of the spinal cord as compared with the wild-type control. The temporal and spatial pattern of GFAP expression was similar to that of F4/80. Taken together these results indicate that expression of CCL21 in the CNS resulted in significant gliosis.

**Expression of chemokines and cytokines in the CNS of the MBPCCL21 mice**

Neutrophils and eosinophils were the primary infiltrating cells in the CNS of the MBPCCL21 mice. However, CCL21 has not been reported to promote chemotaxis of these cell populations (Refs. 17–20 and our unpublished results), suggesting that indirect mechanisms accounted for their presence in the CNS. To identify these mechanisms, we evaluated the levels of chemokines and cytokines in the brains of the transgenic mice. A representative series of four wild-type and 14 transgenic brain homogenates were tested for the expression of the cytokines IL-1α, IL-4, IL-5, TNF-α, and IFN-γ. We did not detect expression of IL-4, IL-5, or IFN-γ in wild-type or transgenic brains (data not shown). However, elevated concentrations of IL-1α and TNF-α were observed in one third of the transgenic brain homogenates, but not in serum, suggesting a localized rather than systemic inflammatory process. Interestingly, a consistent and significant increase was observed in the levels of the

![FIGURE 5. Gliosis in the MBPCCL21 CNS. Paraffin-embedded spinal cord sections of wild-type (A and C) or MBPCCL21 mice (B and D) were stained with F4/80 (A and B) or GFAP (C and D) Abs. Bar = 15 μm in A and B and 20 μm in C and D.](image-url)
chemokines KC, eotaxin, and MIP-1α in the transgenic brain homogenates (Fig. 6).

Spongiosis and hypomyelination in the CNS of MBPCCL21 mice

In addition to leukocyte infiltration and gliosis, mild spongiosis was also observed in the cerebellum, medulla, and spinal cord of P11 and older MBPCCL21 mice (Fig. 7A). Inflammatory cells were often, but not always, found in areas with spongiosis. In the brain, spongiosis was frequently detected in areas associated with control of motor function and balance, such as deep cerebellar nuclei and vestibular nuclei. Ultrastructurally, the spongiosis observed microscopically correlated with a spongiform myelinopathy (Fig. 7, B and C).

Myelin sheaths contained vacuoles and were split in the intraperiod line. Defects in the myelin sheaths often contained irregular whorls of myelin debris. On occasion, axons were dilated and sometimes contained clusters of organelles. However, most axons with myelin alterations appeared normal. A few macrophages containing lipid and myelin debris were adjacent to blood vessels and were scattered throughout the parenchyma, consistent with breakdown and phagocytosis of myelin.

Luxol fast blue staining for myelin demonstrated a decrease in the intensity of staining in brain and spinal cord of MBPCCL21 mice, compared with wild-type, at about P14 and older ages (Fig. 8, A and B), suggesting hypomyelination. The white matter of the spinal cord, cerebellum, and medulla were most severely affected. TUNEL staining was used next to investigate whether the apoptosis of oligodendrocytes could account for the hypomyelination observed in MBPCCL21 mice. Brain sections taken from mice P14 and older showed that the number of apoptotic cells in the transgenic mice was slightly higher than in their littermates (data not shown). However, the majority of these apoptotic cells were not associated with the white matter, where hypomyelination was most significant. Therefore, oligodendrocyte cell death may not be the primary factor causing hypomyelination.

Discussion

Our study was designed to investigate the ability of two lymphocyte chemoattractant chemokines (CCL19 and CCL21) to promote influx of lymphocytes into the CNS. To this end, we expressed these transgenes in the oligodendrocytes. This approach has been used to investigate the function of other chemokines (44). In this study we show that constitutive expression of the chemokine CCL21 in the CNS leads to the development of a severe neuro-pathological condition and death in the majority of the animals. The earliest abnormality in the CNS of these transgenic mice was astrocyte activation, as indicated by increased expression of the glial cell marker GFAP in the spinal cord at P8. The up-regulation of GFAP preceded the onset of neurological signs, which were first detected at P9–P10, and the onset of neurological signs preceded

**FIGURE 6.** Elevated levels of chemokines in the MBPCCL21 brain homogenates. Bars represent average ± SE. *, p < 0.05.

**FIGURE 7.** Spongiosis and myelination defects in MBPCCL21 brains. A, Spongiosis in the neuropil below the cerebellum (molecular layer of cerebellum at upper left). Myelin can be seen within some of the vacuoles, sometimes appearing to encircle axons. Bar = 25 μm. Transmission electron micrographs of the brain of a MBPCCL21 mouse show myelination defects (B and C). B, Vacuoles within myelin sheaths. Note the axons (arrows) appear normal. Several other myelinated axons that lack vacuolation are also present. Bar = 1.3 μm. C, Higher magnification from a different area, showing myelin splitting (arrow) at the intraperiod line. A longitudinal section of the axon (+) is on the left, and a portion of an oligodendrocyte is in the upper right. There is myelin debris (arrowhead) in the interlamellar space. Bar = 0.19 nm.
the activation of microglia, spongiosis, and inflammatory infiltrates, first detected at P10–P12. In contrast, animals expressing the closely related chemokine CCL19 did not show any phenotypic abnormality.

The phenotypic differences observed between MBPCCL21 and MBPCCL19 transgenic mice are very intriguing because these two chemokines are not only functionally related but also can bind to the same two cell surface receptors, CCR7 and CCR11 (25, 31, 45, 46). Because expression of CCL19 did not lead to any neurological phenotype, it is reasonable to suspect that the neurological phenotype observed in the MBPCCL21 mice could be due to the interaction of CCL21 with a receptor other than CCR7 and CCR11 in the CNS. One such receptor could be CXCR3. Murine CCL21, but not murine CCL19 or human CCL21, binds to CXCR3 (26, 27). Furthermore, expression of CXCR3, but not CCR7, has been detected in ischemic mouse brain, cultured microglia, cultured primary astrocytes, and normal and diseased human CNS (47–49). CCL21 is not expressed normally in the CNS, but its expression has been reported in ischemic brain (48). It has also been shown that CCL21 induces chemotaxis and intracellular calcium mobilization in cultured microglia, and that these effects can be cross-desensitized by a ligand for CXCR3, CXCL10 (48). These observations have led to the suggestion that CCL21 and CXCR3 may mediate neuron-glia interactions during disease conditions. Thus, CCL21 expressed by oligodendrocytes may have interacted with CXCR3 expressed in astrocytes and/or microglia, inducing gliosis and production of inflammatory mediators including cytokines and chemokines, which in turn may have facilitated the influx of inflammatory cells into the CNS. Indeed, we have observed production of inflammatory cytokines and of neutrophil and eosinophil chemoattractant chemokines in the MBPCCL21 brains (Fig. 6). The expression of these chemokines may have been important to promote the influx of eosinophils and neutrophils into the CNS. Influx of these leukocytes into the CNS may have also contributed to the spongiosis and hypomyelination observed in the MBPCCL21 mice. Eosinophils, for instance, have been shown to contain a neurotoxin that causes spongiosis in the white matter of the cerebellum, medulla, and spinal cord following intracerebral or intrathecal injection (50, 51). Moreover, eosinophils have been found in the spinal cord of mice with experimental autoimmune encephalomyelitis and are suspected to play a role in nervous system damage observed in experimental autoimmune encephalomyelitis (52, 53). Alternatively, expression of CCL21 may have induced gliosis and disease by a receptor-independent mechanism, such as direct toxicity of oligodendrocytes (54–61).

The paradoxical lack of lymphocyte infiltration in tissues expressing potent lymphocyte chemoattractants is arguably the most provocative finding of this study. We first considered the possibility that the activation state of the endothelium could have influenced entry of lymphocytes responding to the CCL19 or CCL21 produced in the CNS. Lymphocyte entry into the CNS has been shown to depend on the activation of the endothelium by several cytokines (62), IFN-γ and TNF-α have been shown to activate brain endothelium in vitro (63), and transgenic mice expressing IFN-γ and TNF-α present lymphocytic infiltrates (60, 64). Interestingly, despite elevated levels of TNF-α in some of the MBPCCL21 brains, we failed to detect lymphocytes in the CNS parenchyma or in the perivascular space. Other parameters of endothelial function, such as the permeability of the blood-brain barrier (BBB) were also considered, because the integrity of the BBB has been shown to be yet another parameter regulating entry of the lymphocytes into the CNS (65). Immunohistochemical staining with an anti-mouse IgG Ab of the MBPCCL21 brains showed that the BBB was disrupted in areas heavily infiltrated with PMNs (data not shown), but no lymphocytes were detected in these areas. Finally, we considered the possibility that high levels of CCL19 or CCL21 may have prevented lymphocyte influx. The current paradigm postulates that leukocytes migrate toward a chemokine gradient and that high chemokine concentrations prevent further migration (for review, see Ref. 9). Thus, high levels of CCL19 or CCL21 in the transgenic brains could actually have prevented, rather than promoted, lymphocyte migration. However, no lymphocytes were observed in the CNS of transgenic mice expressing low levels of CCL19 or CCL21. Thus, it is unlikely that high levels of CCL19 or CCL21 explain the lack of lymphocyte infiltration in these models.

In summary, expression of the T cell chemoattractant chemokine CCL21 in the CNS led to the development of a striking neurological phenotype and premature death. The pathological findings in the CNS included reactive gliosis, spongiosis, hypomyelination, and parenchymal infiltration by polymorphonuclear cells but, surprisingly, no lymphocytic infiltration. Lack of lymphocyte infiltration was also observed in mice expressing the closely related molecule CCL19. In light of these findings, we conclude that 1) CCL21 is not sufficient to promote lymphocyte influx into the CNS under basal conditions (as can be appreciated by the analysis of the transgenic mice expressing low levels of CCL21), 2) CCL21 is not sufficient to promote lymphocyte influx into the CNS under inflammatory conditions (as can be appreciated by the analysis of the transgenic mice expressing high levels of CCL21), and 3) the closely related chemokine CCL19 does not promote lymphocyte recruitment into the CNS. Fan et al. (66) have recently shown that ectopic expression of CCL21 in the pancreas leads to the formation of lymphoid structures. We have also found that expression of CCL21 in the pancreas induces recruitment of lymphocytes and formation of lymph node-like structures, but that this effect cannot be reproduced when CCL21 is overexpressed in the skin (67). Taken together these results suggest that CCL21 may...
require a tissue-specific environment to induce lymphocyte recruitment and accumulation. These requirements may include the expression of specific adhesion molecules, or cofactors which facilitate lymphocyte recruitment and lymphoid neogenesis. The nature of these requirements is unknown at present, but they are clearly satisfied in lymphoid tissues and in transgenic pancreas when CCL21 is ectopically expressed. It will be interesting to define whether ectopic expression of CCL19 in pancreas will also result in lymphocyte accumulation and lymphoid neogenesis.

In conclusion, the results reported in this work and those by Chen et al. (67) reveal a novel finding in chemokine biology, namely that there are tissue-specific requirements for lymphocyte recruitment induced by a single chemokine. Defining the nature of these requirements will hopefully contribute to a better understanding of the role of chemokines in leukocyte trafficking and lymphoid neogenesis.

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