Cutting Edge: The B Cell Chemokine CXC Chemokine Ligand 13/B Lymphocyte Chemoattractant Is Expressed in the High Endothelial Venules of Lymph Nodes and Peyer's Patches and Affects B Cell Trafficking Across High Endothelial Venules

Yukihiko Ebisuno, Toshiyuki Tanaka, Naotoshi Kanemitsu, Hidenobu Kanda, Kazuhiro Yamaguchi, Tsuneyasu Kaisho, Shizuo Akira and Masayuki Miyasaka

*J Immunol* 2003; 171:1642-1646; doi: 10.4049/jimmunol.171.4.1642

http://www.jimmunol.org/content/171/4/1642

Supplementary Material  http://www.jimmunol.org/content/suppl/2003/08/01/171.4.1642.DC1

References  This article *cites 17 articles*, 9 of which you can access for free at: http://www.jimmunol.org/content/171/4/1642.full#ref-list-1

Subscription  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Cutting Edge: The B Cell Chemokine CXC Chemokine Ligand 13/B Lymphocyte Chemoattractant Is Expressed in the High Endothelial Venules of Lymph Nodes and Peyer’s Patches and Affects B Cell Trafficking Across High Endothelial Venules

Yukihiko Ebisuno,† Tsuyoshi Tanaka,‡† Naotoshi Kanemitsu,*, Hidenobu Kanda,*, Kazuhito Yamaguchi,¶ Tsuneyasu Kaisho,‡§ Shizuo Akira,‡ and Masayuki Miyasaka3*

While CCR7 ligands direct T cell trafficking into lymph nodes (LNs) and Peyer’s patches (PPs), chemokines that regulate B cell trafficking across high endothelial venules (HEVs) remain to be fully elucidated. Here we report that CXC chemokine ligand (CXCL)13 (B lymphocyte chemoattractant) is detected immunohistologically in the majority of HEVs in LNs and PPs of nonimmunized mice. Systemically administered anti-CXCL13 Ab bound to the surface of ~50% of HEVs in LNs and PPs, but not to other types of blood vessels, indicating that CXCL13 is expressed in the HEV lumen. In CXCL13-null mice, B cells rarely adhered to PP HEVs, whereas T cells did efficiently. Superfusion of CXCL13-null PPs with CXCL13 restored the luminal presentation of CXCL13 and also B cell arrest in PP HEVs at least partially. Collectively, these results indicate that CXCL13 expressed in the HEV lumen plays a crucial role in B cell trafficking into secondary lymphoid tissues such as PPs. The Journal of Immunology, 2003, 171: 1642–1646.

Accumulating evidence indicates that certain chemokines play a critical role in triggering the integrin-dependent adhesion of lymphocytes to high endothelial venules (HEVs) and their subsequent trafficking into lymph nodes (LNs) and Peyer’s patches (PPs). In particular, CXC chemokine ligand (CCL)21 (secondary lymphoid tissue chemokine) and CCL19 (EBI1 ligand chemokine) are highly localized to HEVs and their subsequent trafficking into lymph nodes (LNs) and Peyer’s patches (PPs) (1). In CXCL13-deficient mice, B cells rarely adhered to PP HEVs, whereas T cells did efficiently. Superfusion of CXCL13-null PPs with CXCL13 restored the luminal presentation of CXCL13 and also B cell arrest in PP HEVs at least partially. Collectively, these results indicate that CXCL13 expressed in the HEV lumen plays a crucial role in B cell trafficking into secondary lymphoid tissues such as PPs. The Journal of Immunology, 2003, 171: 1642–1646.

Materials and Methods

Animals

C57BL/6 mice were purchased from SLC (Hamamatsu, Japan) and housed under specific pathogen-free conditions. CXCL13-deficient mice were generated by replacing the coding region between the 24th and 66th amino acids with the inverted neomycin resistance gene (T. Kaisho and S. Akira, unpublished observations). The CXCL13-deficient mice were backcrossed to C57BL/6 mice at least four times and used. This CXCL13-null mouse strain exhibits the same phenotype as that previously reported (10). The experimental protocol was approved by the Animal Care Committee of Osaka University.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Advanced Research on Cancer from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a grant for Advanced Research on Cancer from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Received for publication January 7, 2003. Accepted for publication June 19, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*Laboratory of Molecular and Cellular Recognition, Osaka University Graduate School of Medicine, Suita, Japan; †Institute of Laboratory Animals, Yamaguchi University School of Medicine, Ube, Japan; ¶Research Institute for Microbial Diseases, Osaka University, Suita, Japan; and §§Research Center for Allergy and Immunology, The Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan.

Copyright 2003 by The American Association of Immunologists, Inc.
was approved by the Ethics Review Committee for Animal Experimentation of Osaka University Medical School.

Antibodies
Polyclonal anti-mouse CXCL13 Abs (M-17 and V-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Dot blot analysis indicated that these Abs reacted specifically with mouse CXCL13 and failed to cross-react with human and mouse CCL21, human CCL19, or human CXCL13 (data not shown). Amino acid sequencing of the immunizing peptides (Santa Cruz Biotechnology) revealed that the M-17 and V-20 Abs were raised against the mouse CXCL13 peptides KSLSTQAPVXKRAA (93–109 aa) and VNPRAXWLQRLRHIQV (76–91 aa), respectively. Biotinylated anti-mouse CCL19, anti-mouse CCL21 and anti-mouse CXCL13 Abs were obtained from DAKO (Glostrup, Denmark), PeproTech (Rocky Hill, NJ) and Santa Cruz Biotechnology, respectively. MECA-79 (anti-peripheral node addressin (PNAd)) and MEC-367 (anti-mucosal addressin cell adhesion molecule (MAdCAM)-1) were kindly provided by Dr. E. C. Butcher (Stanford University, Stanford, CA) and used as described (11, 12). Biotin-conjugated mAbs to CD3, B220, and CD11b were from BD PharMingen (San Diego, CA).

Immunohistochemical analysis
Immunostaining of frozen sections was performed as previously described (13). Bound Abs were detected with biotinylated secondary Abs, followed by alkaline phosphatase-conjugated avidin-biotin-complex reagent (Vector Laboratories, Burlingame, CA) and Vector Red (Vector Laboratories). For double staining, the sections were further incubated with FITC– or Alexa Fluor 488 (Molecular Probes, Eugene, OR)-conjugated MECA-79 or MECA-367. To determine the proportion of chemokine-expressing HEVs, the number of HEVs positively stained with anti-chemokine Abs were counted. Approximately 100 randomly selected PNAd+ and MAdCAM-1+ HEVs were examined for each chemokine.

In situ staining analysis
Anti-CXCL13 Ab (M-17) or control goat IgG (50 μg/ml per mouse) was administered by intracardiac injection into mice. Ten minutes after the injection, the mice were perfused with 4% paraformaldehyde, and their mesenteric LNs and intestinal tissues were harvested. The number of lymphocytes that had migrated into these tissues was determined by intracardiac injection into mice. Ten minutes after the injection, the mice were perfused with 4% paraformaldehyde, and their mesenteric LNs and intestinal tissues were harvested. The number of lymphocytes that had migrated into these tissues was determined by flow cytometry using an EPICS-XL flow cytometer (Beckman Coulter, Fullerton, CA). Because the cells were brightly labeled with 5-TRITC, fluorescence from 5-TRITC, which was excited by the argon laser (488-nm), was successfully detected by the photomultiplier FL2. Although a significant amount of 5-chloromethylfluorescein diacetate fluorescence was also collected by the FL2 detector, it could be easily distinguished from 5-TRITC fluorescence by the difference in intensity.

CXCL13 is expressed in the majority of HEVs
While looking for genes specifically expressed in HEVs (11, 12) we noticed that transcripts for a B cell chemokine CXCL13/BLC were found in low abundance in cDNA libraries that we generated from PNAd+ and MAdCAM-1+ HEVs. Therefore, we performed immunohistochemical analyses to examine whether CXCL13 is expressed in HEVs at the protein level. As shown in Fig. 1, the anti-peptide Ab against mouse CXCL13 (M-17) reacted strongly with MAdCAM-1+ LN HEVs from wild-type mice (Fig. 1, top panels); this reaction was almost completely abrogated by the addition of a 100-fold excess (in molar ratio) of the M-17 Ag peptide used to generate the M-17 Ab, but not by the V-20 peptide (Fig. 1, middle panels). Further verifying the specificity of this Ab, M-17 failed to react with any cell components in LN sections obtained from the CXCL13-deficient mice (Fig. 1, bottom panels). These results strongly indicate that the CXCL13 protein is expressed in MAdCAM-1+ LN HEVs. Furthermore, as shown in Fig. 2, the anti-CXCL13 Ab reacted with the majority of PNAd+ HEVs as well as MAdCAM-1+ HEVs in the LNs and PPs, although the extent of the reactivity was variable in different HEV segments. In addition, an anti-CCL21 Ab also reacted with over 80% of the HEVs, suggesting that both CXCL13 and CCL21 are expressed simultaneously in the majority of HEVs in these lymphoid tissues. In contrast, an anti-CCL19 Ab reacted with ~50% of the HEVs in LNs and PPs, in line with the observation in human tonsils (3). In addition, an anti-CXCL12 Ab also bound to a considerable proportion of HEVs in LNs and PPs (data not shown) as previously reported (6).

CXCL13 is apparently expressed in the luminal surface of HEVs
We next addressed whether CXCL13 is present in the luminal surface of HEVs in a form that is accessible to the receptor-bearing lymphocytes. Intracardiac injection of the anti-CXCL13 M-17 Ab yielded distinct binding to the luminal surface of

![Image](https://example.com/image.jpg)

**FIGURE 1.** Immunohistochemical analysis of CXCL13 expression in LN HEVs. Top panels, Dual staining of mesenteric LNs from wild-type mice with Abs to CXCL13 (M-17) (red) and MAdCAM-1 (green). Middle panels, Competitive inhibition of anti-CXCL13 (M-17) binding with the M-17 peptide or irrelevant V-20 peptide. Bottom panels, Dual staining of mesenteric LNs from CXCL13-null mice. Sections were doubly stained with Abs to CXCL13 and MAdCAM-1 as above. Note the distinct CXCL13 staining in MAdCAM-1+ HEVs in the wild-type mice but the lack of such staining in CXCL13-null mice. Original magnification, ×400

The on-line version of this article contains supplemental material.
Despite the strong expression of the CXCL13 protein in HEVs, only low levels of CXCL13 mRNA were detected by in situ hybridization (data not shown), suggesting that CXCL13 mRNA may be expressed by HEVs only weakly if at all. Certain chemokines such as CCL19, which is produced by nonendothelial stromal cells in lymphoid tissues, have been shown to be delivered to and presented by HEVs (3). Therefore, it is conceivable that CXCL13 protein expressed in HEVs is produced in some non-HEV region(s) within the lymphoid tissue and subsequently transported to HEVs. Follicular dendritic cells are certainly a possible source of CXCL13, because they have been shown to produce it (7). The derivation of the CXCL13 protein detected in the HEVs needs to be determined in future investigation.

**B cell adhesion occurs only poorly in HEVs in CXCL13-deficient mice**

We next asked whether CXCL13 is involved in B cell adhesion to HEVs. To this end, we i.v. injected into wild-type or CXCL13-deficient mice B cells and T cells that had been labeled with different fluorescent dyes and monitored the behavior of these cells within the PPs by intravital microscopy. In the wild-type mice, the injected B and T cells both showed rolling along and firm adhesion to the endothelium of HEVs in the PPs as expected (Fig. 4, A and B, top panels). As has been reported by Warnock et al. (16) and Miura et al. (17), some of the injected B and T cells bound differentially to distinct HEV segments. At the same time, a substantial proportion of the cells accumulated in the same HEV regions, in line with the above observation that both CCL21 and CXCL13 are expressed in the majority of HEVs. In fact, Warnock et al. (16) also described the presence of HEVs that allowed adhesion of T and B cells. In CXCL13-null mice (Fig. 4, A and B, middle panels), however, where HEVs were much less abundant than in wild-type controls, it was notable that B cells rolled well but adhered poorly to the HEV segments, whereas T cells rolled and adhered efficiently, as was observed in the wild-type mice. The apparently uncompromised behavior of T cells in the CXCL13-null environment was expected, given that the expression of other HEV-associated chemokines including CCL21 and CXCL12 in the HEVs of CXCL13-deficient mice was unaffected (data not shown). When short-term homing assays of purified T and B cells were performed, we found that B cells migrated into the mesenteric LNs of the CXCL13-deficient mice at a reduced level than in normal mice, whereas they migrated into the spleen of the CXCL13-deficient and wild-type mice at comparable levels (Fig. 4C). In contrast, coinjected T cells migrated equally well into the mesenteric LNs, as well as the spleen, in normal and CXCL13-deficient mice (Fig. 4C). These results are compatible with the above results obtained by intravital microscopy.

**Reconstitution of CXCL13 restores B cell sticking in CXCL13-null PP HEVs**

Because it may be argued that CXCL13-deficiency could induce indirect defects in B cell sticking to HEVs observed in BLC-null mice, we next investigated whether reconstitution of CXCL13 expression in CXCL13-deficient HEVs could induce B cell sticking. Upon superfusion of PPs with CXCL13, MadCAM-1+ HEVs of PPs of CXCL13-deficient mice restored CXCL13 expression as evidenced by the appearance of in situ staining with anti-CXCL13 Ab (Fig. 4D), indicating that CXCL13 exogenously administered by superfusion was transported to the luminal surface of PP HEVs. When fluorescently labeled lymphocytes were injected i.v. subsequent to the
CXCL13 superfusion, not only T cells but also B cells adhered to PP HEVs of CXCL13-deficient mice (Fig. 4, A and B, bottom panels), while B cell sticking was less abundant than that seen in wild-type mice. There results demonstrate that forced expression of CXCL13 restored B cell sticking to PP HEVs at least partially in CXCL13-deficient mice.

Our results are consistent with the observation by Okada et al. (6) that signals through CXCR5 are critically important to B cell sticking to PP HEVs. The present study also extends their finding to show that CXCL13, a physiological ligand for CXCR5, is essential for B cell adhesion to certain HEV segments in PPs. However, with regard to the LN HEVs, the CXCL13 expression and its role in B cell homing reported in the present study are at variance with the previous report by Okada et al. (6). In this regard, it should be noted that we used an anti-CXCL13 Ab (M-17) that is different from what Okada et al. used in immunohistochemical analysis. The anti-CXCL13 Ab (M-17) gave heterogeneous but significant staining in different HEVs and a different anti-CXCL13 Ab (V-20) that recognizes a different amino acid sequence from M-17 gave only faint staining in the HEVs (data not shown), possibly indicating that not all epitopes of CXCL13 in HEVs are presented equally on HEVs. Some epitopes of CXCL13 may be more readily detected than others. In addition, we examined B cell homing into mesenteric LNs at a time point that is different from what Okada et al. used; we analyzed B cell migration at 6 h after B cell injection, whereas Okada et al. investigated it at 90 min after B cell injection. These differences in the experimental conditions may, at least in part, account for the apparent discrepancy between the two studies.

In conclusion, the present investigation demonstrates that CXCL13 is expressed in the luminal surface of HEVs in LNs and PPs and plays a crucial role in B cell trafficking across HEVs. Further investigation of the mechanisms of B cell trafficking across HEVs is now warranted, which should lead to a more precise understanding of the molecular cues that guide B cells to appropriate sites.

Acknowledgments
We thank Dr. E. C. Butcher for the MECA-79 and MECA-367 mAbs. We also thank Drs. T. Murai and T. Hirata for critical reading of the manuscript, T. Kondo for technical help, and S. Yamashita and M. Komine for secretarial assistance.

References