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Cutting Edge: GPR35/CXCR8 Is the Receptor of the Mucosal Chemokine CXCL17

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Chemokines are chemotactic cytokines that direct the traffic of leukocytes and other cells in the body. Chemokines bind to G protein–coupled receptors expressed on target cells to initiate signaling cascades and induce chemotaxis. Although the cognate receptors of most chemokines have been identified, the receptor for the mucosal chemokine CXCL17 is undefined. In this article, we show that GPR35 is the receptor of CXCL17. GPR35 is expressed in mucosal tissues, in CXCL17-responsive monocytes, and in the THP-1 monocyteid cell line. Transfection of GPR35 into Ba/F3 cells rendered them responsive to CXCL17, as measured by calcium-mobilization assays. Furthermore, GPR35 expression is downregulated in the lungs of Cxcl17−/− mice, which exhibit defects in macrophage recruitment to the lungs. We conclude that GPR35 is a novel chemokine receptor and suggest that it should be named CXCR8. The Journal of Immunology, 2015, 194: 29–33.

Chemokines (chemotactic cytokines) are small secreted proteins that direct the migration of various cell types (1). They have been classified as homeostatic or inflammatory, depending on the stimuli that regulate their production (2). Chemokines bind receptors that belong to the class A G protein–coupled receptor (GPCR) superfamily. Binding these receptors triggers signaling cascades that promote multiple cellular functions (3) through the coupling of G proteins. The human chemokine superfamily includes 48 ligands and 19 receptors. The receptors for most of the ligands have been identified (2), and only two chemokine ligands remain orphan (i.e., their receptors have not been identified: CXCL14 and CXCL17). CXCL17 was the last chemokine described (4), and its expression pattern is closely associated with mucosal tissues (5, 6). Few reports exist on CXCL17, but it is known to chemoattract macrophages both in vitro (4, 6), and in vivo (7). CXCL17 is also known to promote angiogenesis (6).

In this article, we show that CXCL17 signals through the orphan GPCR GPR35. This receptor is not known to bind chemokines (8); however, like CXCL17, it exhibits a mucosal expression pattern (9). Partly because of this, it has attracted attention as a potential therapeutic target (9). Because our findings indicate that it represents a novel chemokine receptor, we suggest that it should be named CXCR8.

Materials and Methods

Cells and reagents

THP-1 leukemia cells and the pro-B cell line Ba/F3 were maintained in RPMI 1640. Abs used include rabbit IgG (Jackson ImmunoResearch, West Grove, PA) and polyclonal rabbit anti-human GPR35 (Cayman Chemicals, Ann Arbor, MI). A clone encoding human GPR35 was obtained from The Missouri S&T cDNA Resource Center, under GenBank accession number AY275467 (http://www.ncbi.nlm.nih.gov/nuccore/AY275467).

Body Index of Gene Expression database

The Body Index of Gene Expression is a comprehensive database of human gene expression (5, 10). Data from a probeset (210264.at) corresponding to GPR35 were used to determine its expression in the database.

Quantitative real-time PCR analysis

Quantitative real-time PCR (qRT-PCR) data were generated with a LightCycler 480 (Roche). cDNA was obtained from total RNA extracted from THP-1 cells using QIAGEN kits. Gene-specific primers and corresponding universal probes were used to quantify GPR35 or control gene transcripts.

Chemotaxis assays

Chemotaxis assays were performed for 18–20 h using 5.0-μm, 24-well Transwell migration plates (Corning, Manassas, VA), with 200 ng/ml chemokine (R&D Systems) in 600 μl incomplete RPMI added to the bottom chambers (0.5–1.0 × 106 cells/well). Where noted, cells were pretreated with 200 ng/ml Bordetella pertussis toxin (PTX) or 10 μM PGE2 (both from Sigma-Aldrich, St. Louis, MO) for 24 h.

Quantitation of chemotaxis by flow cytometry

This protocol was adapted from Proudfoot et al. (11). Briefly, the chemotaxed cells were resuspended in 200 μl 1× PBS. Standards were generated through 10-fold dilutions ranging from 106 to 102 cells/200 μl. The cell counts were the number of events in 30 s recorded in a FACSCalibur (Becton Dickinson).

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Abbreviations used in this article: GPCR, G protein–coupled receptor; PTX, Bordetella pertussis toxin; qRT-PCR, quantitative real-time PCR; WT, wild-type.

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GPR35-transfection assays
A total of $2 \times 10^7$ cells/ml Ba/F3 cells was resuspended in 500 µl cytomix (12) and transferred to a 0.4-cm electroporation cuvette (USA Scientific). Then, 20 µg pcDNA3.1+/GPR35 DNA was added prior to electroporation using a Bio-Rad system (300 V, 960 µF). Cells were cultured in RPMI 1640 at 37°C for 48 h before performing assays.

Calcium-mobilization assays
A total of $5 \times 10^7$ THP-1 or Ba/F3 cells/ml was loaded with Calcium Green-1, AM and Fura Red, AM (Life Technologies, Carlsbad, CA) at 10 µmol/l for 30 min at 37°C. After 30 s, cells were stimulated with human CXCL17 (R&D Systems) or 100 µM ionomycin (Sigma-Aldrich; positive control). The Calcium Green/Fura Red fluorescence ratio was measured in a FACS-Calibur before and after the addition of activators and was analyzed with FlowJo software.

Wild-type and Cxcl17−/− mice
Lung tissue was collected from wild-type (WT) C57BL/6 or Cxcl17−/− mice, which were obtained as described (13). All mouse studies were performed under a protocol approved by the Institutional Animal Care and Use Committee of the University of California Irvine.

Results and Discussion
We report a novel chemokine receptor, GPR35, which we identified as the receptor of CXCL17, a chemokine first reported in 2006 (4). CXCL17 is a mucosal chemokine that has been associated with human diseases, including idiopathic pulmonary fibrosis (5) and cancer (14, 15). Recently, we reported that Cxcl17−/− mice exhibit a paucity of macrophages in the lungs (7), whereas, conversely, injection of Cxcl17 i.p. induces strong macrophage recruitment in vivo (7). In vitro, macrophages (including THP-1 cells) chemotax to CXCL17, as well as to CCL2 (data not shown), a well-known macrophage chemoattractant (16). These data indicate that CXCL17 is a major macrophage chemoattractant.

CXCL17 induces chemotactic responses in the THP-1 human monocyte cell line (Fig. 1A), indicating that THP-1 expresses the CXCL17 receptor. The chemotactic response of THP-1 to CXCL17 was amplified by pretreatment with PGE2 (Fig. 1A), as was shown for other chemokines (17). These responses are also sensitive to PTX (Fig. 1A), which inhibits Gαi/o protein–signaling pathways, as observed for other chemokine receptors (18).

The binding of chemokines to their receptors induces an increase in cytosolic calcium (18). As shown in Fig. 1B, CXCL17 induces a Ca2+ flux in both resting and PGE2-treated cells; however, as observed with chemotactic responses (Fig. 1A), the Ca2+ fluxes observed in PGE2-treated THP-1 cells in response to CXCL17 were stronger (Fig. 1B). Both CCL2 and CXCL17 induced calcium fluxes in THP-1 cells.

Chemokine receptors can be desensitized for a certain period of time following activation (19). As shown in Fig. 1C, CXCL17 desensitizes itself in THP-1 cells, but it does not desensitize the Ca2+ flux induced by CCL2 (a chemokine that signals through CCR2) (Fig. 1C) (20). Conversely, CCL2 did not desensitize CXCL17-mediated responses, indicating that these two chemokines signal through different receptors (19).

Before searching for the CXCL17 receptor, we tested whether CXCL17 bound or activated other known chemokine receptors. We confirmed that CXCL17 does not bind or signal to CCR2, CCR5, CXCR2, CXCR3, CXCR4, or CXCR7 (Supplemental Tables 1A, 1B). Therefore, we decided to search for orphan GPCRs expressed in macrophages as potential candidates to be the CXCL17 receptor.

We screened the Body Index of Gene Expression database of human gene expression (10) to identify GPCRs expressed by monocytes. We narrowed the list of possible candidates by selecting orphan GPCRs that exhibit structural characteristics of chemokine receptors and a tissue-expression pattern similar to CXCL17. The orphan receptor GPR35 is known to be
expressed in mucosal tissues, including the gastrointestinal tract (21) and adult lung (22), and this expression profile correlates strongly with the expression of CXCL17. Importantly, it is also expressed by monocytes (23). The ImmGen database (www.immgen.org) indicates that GPR35 is expressed by dendritic cells, macrophages, and granulocytes, all of which chemotax to CXCL17 (4, 24).

To assess the ability of CXCL17 to signal through GPR35, we sought to induce a CXCL17-mediated calcium flux in previously nonresponsive cells by transfecting this receptor into a GPR35-null cell line. We transfected the mouse pro-B cell line Ba/F3, which does not express GPR35 (data not shown) (25). CXCL17 induced a robust calcium flux in GPR35-transfected BA/F3 cells (Fig. 2A), and it exhibited a dose-response pattern with increasing CXCL17 concentrations (Fig. 2B). Another orphan chemokine, CXCL14, did not induce calcium fluxes in GPR35-transfected BA/F3 cells (data not shown). Furthermore, transfection of HEK293 cells also induced a CXCL17-mediated calcium flux (Fig. 2C). GPR35 is located in human chromosome 2 near CXCR7, and a phylogenetic analysis of chemokine receptors indicates that it is closest to CXCR7, suggesting that these genes may share a common ancestor (Supplemental Fig. 1). GPR35 exhibits a DRY box (Fig. 2D), a critical motif shared by all signaling chemokine receptors that is required for G protein coupling (26). GPR35 also exhibits a conserved Asp residue, a Thr-Xaa-Pro motif, and a Cys-Trp-Xaa-Pro motif (27). These regions are highly conserved in chemokine receptors (28, 29). Additionally, some specific residues, known as "micro-switches," which are also highly conserved in chemokine receptors, are present in GPR35 (27). Taken together, these structural characteristics are consistent with the conclusion that GPCR35 is a signaling receptor for CXCL17.

Kynurenic acid, 2-Acyl lysophosphatidic acid, and Zaprinast were reported to be GPR35 agonists (30); however, the responses observed with these ligands are in the micromolar range. In contrast, CXCL17 induces calcium fluxes in the nanomolar range (Fig. 2B), within physiologic concentrations of this chemokine in vivo (31).

We were able to increase the responsiveness of THP-1 cells to CXCL17 through pretreatment with PGE2 (Fig. 1A, 1B), suggesting that PGE2 induces the expression of the CXCL17 receptor in THP-1 cells. Using qRT-PCR, we confirmed that GPR35 expression is significantly elevated in PGE2-treated THP-1 cells (Fig. 3A). We recently reported that a Cxcl17−/− mouse exhibits a paucity of macrophages in the lungs (7), and Cxcl17 chemoattracts macrophages from lungs of WT, but not Cxcl17−/−, mice (7). These observations indicate that CXCL17 is a physiologic signal that recruits certain macrophage subsets to the lungs that should express the CXCL17 receptor. This model predicts that cells expressing the CXCL17 receptor would not be recruited into the lungs of...
Cxcl17−/− mice. To test this prediction, we measured the expression of GPR35 in the lungs of WT and Cxcl17−/− mice. As shown in Fig. 3B, GPR35 is robustly expressed in WT mouse lungs but is strongly downregulated in Cxcl17−/− mouse lungs. This effect is macrophage specific, because there is a concurrent decrease in the expression of two lung macrophage-specific markers in the lungs of Cxcl17−/− mice (7). Taken together, these observations strongly support the conclusion that GPR35 is the receptor of CXCL17. Therefore, we propose renaming GPR35 as CXCR8, in accordance with the chemokine receptor nomenclature (32).

The identification of CXCR8 is an important addition to the chemokine field (33). Importantly, GPR35 already was identified as a target for gastrointestinal diseases (21). Genome-wide association studies linked GPR35 to primary sclerosing cholangitis and ulcerative colitis (34). In the respiratory system, CXCL17 is upregulated in idiopathic pulmonary fibrosis (5). Given the importance of macrophages in inflammation and the strong expression of both CXCL17 (5) and GPR35/CXCR8 in mucosal tissues (21, 22), we hypothesize that the CXCL17/CXCR8 axis is an important player in mucosal inflammation and in the pathology of various human diseases.

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Disclosures
The authors have no financial conflicts of interest.

References


