Cutting Edge: The Orphan Chemokine Receptor G Protein-Coupled Receptor-2 (GPR-2, CCR10) Binds the Skin-Associated Chemokine CCL27 (CTACK/ALP/ILC)

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We recently reported the identification of a chemokine (CTACK), which has been renamed CCL27 according to a new systematic chemokine nomenclature. We report that CCL27 binds the previously orphan chemokine receptor GPR-2, as detected by calcium flux and chemotactic responses of GPR-2 transfectants. We renamed this receptor CCR10. Because of the skin-associated expression pattern of CCL27, we focused on the expression of CCL27 and CCR10 in normal skin compared with inflammatory and autoimmune skin diseases. CCL27 is constitutively produced by keratinocytes but can also be induced upon stimulation with TNF-α and IL-1β. CCR10 is not expressed by keratinocytes and is instead expressed by melanocytes, dermal fibroblasts, and dermal microvascular endothelial cells. CCR10 was also detected in T cells as well as in skin-derived Langerhans cells. Taken together, these observations suggest a role for this novel ligand/receptor pair in both skin homeostasis as well as a potential role in inflammatory responses. The Journal of Immunology, 2000, 164: 3465–3470.

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vector, pMX-CD8-myc. Transfectants underwent selection in G418 and were further enriched by FACS-sorting using an anti-myc Ab (PharMingen, San Diego).

Calcium mobilization assays
To identify the CCL27 receptor, we tested a panel of known human (CCR1, 2, 3, 4, 5, 6, 7, 8, 9, XCR1; CX3CR, CXCR1, 2, 3, 4, 5) chemokine and orphan (STRL33, GPR-15, GPR-2) GPCR transfectants for signaling using a calcium mobilization assay as described previously (9). Mouse CCL27 was obtained from R&D Systems (Minneapolis, MN), and human CCL27 was chemically synthesized by Gryphon Sciences (South San Francisco, CA).

Transwell chemotaxis assay
The transwell chemotaxis assays were performed using 10^6 BAF/3-hCCL10(GPR-2) transfectant or parental cells as previously described (4).

Cell isolation and cell culture
Human primary epidermal keratinocytes, dermal fibroblasts, melanocytes, and dermal microvascular endothelial cells were purchased from Clonetics (San Diego, CA) and cultured in keratinocyte, fibroblast, melanocyte, or dermal microvascular endothelial cells were purchased from Clonetics (San Diego). Human primary epidermal keratinocytes, dermal fibroblasts, melanocytes, and dermal microvascular endothelial cells were purchased from Clonetics (San Diego). Human primary epidermal keratinocytes, dermal fibroblasts, melanocytes, and dermal microvascular endothelial cells were purchased from Clonetics (San Diego). Human primary epidermal keratinocytes, dermal fibroblasts, melanocytes, and dermal microvascular endothelial cells were purchased from Clonetics (San Diego).

Biopsy samples
Six-millimeter punch biopsies were taken, after obtaining informed consent, from either lesional skin of patients with psoriasis (n = 21), atopic dermatitis (n = 10), or cutaneous lupus erythematosus (n = 10), or from normal (n = 10) healthy individuals. Skin samples were immediately frozen in liquid nitrogen and stored at −80°C. This study was approved by local ethics committees.

Real-time quantitative PCR (TaqMan) analysis of CCL27 and CCR10 mRNA expression
RNA from both homogenized skin samples or human cells was extracted using RNA STAT 60 according to the manufacturer's protocol (Tel-Test, Friedensburg, TX). Four micrograms of RNA were treated with DNase I (Boehringer Mannheim, Mannheim, Germany) and reverse transcribed with oligo dT14–18 (Life Technologies, Gaithersburg, MD) and random hexamer primers (Promega, Madison, WI) using standard protocols. cDNA was diluted to a final concentration of 10 ng/μl. cDNA was analyzed for the expression of human CCL27 and CCR10 (GPR-2) genes by the fluorogenic 5'-nuclease PCR assay (13) using a Perkin-Elmer ABI Prism 7700 Sequence Detection System (Perkin-Elmer, Foster City, CA). Briefly, 10 μl cDNA (100 ng) were amplified in the presence of 12.5 μl TaqMan universal master mix (Perkin-Elmer), 0.625 μl gene-specific TaqMan probe, 0.5 μl gene-specific forward and reverse primers, and 0.5 μl water. As an internal positive control, 0.125 μl 18S RNA-specific TaqMan probe and 0.125 μl 18S RNA-specific forward and reverse primers were added to each reaction. Specific primers and probes for CCL27, CCR10, and the other chemokine receptors measured were obtained from Perkin-Elmer. Samples underwent the following stages: stage 1, 50°C for 2 min; stage 2, 95°C for 10 min; and stage 3, 95°C for 15 s followed by 60°C for 1 min. Stage 3 was repeated 40 times. Gene-specific PCR products were measured by means of an ABI PRISM 7700 Sequence Detection System (Perkin-Elmer) continuously during 40 cycles. Standard curves for CCL27 and CCR10 expression were generated amplifying 10-fold serial dilutions of known quantities of CCL27 and CCR10 plasmid DNA. Quantification of target gene expression was obtained using sequence detector system software (Perkin-Elmer). Human cDNA libraries used in this study were generated as described previously (1, 4, 14, 15). For analyses using cDNA libraries, a human GAPDH-specific primer/probe pair was used as internal positive control.

Results
We have previously described the discovery and characterization of a new CC chemokine (CTACK) now designated CCL27. This

FIGURE 1. Amino acid sequence alignment of human CCR10. Amino acid sequences of the original and full-length hGPR-2 are aligned with other chemokine receptors, bCCR8, hCCR9, hCXCR1, and hCXCR2. Dark-shaded boxes indicate identical amino acids. Conserved amino acids are shaded light grey. *, The missing amino acid sequence at the NH2 terminus of the full-length in comparison to the previously published truncated form of hGPR-2. **, The characteristic DRY box motif.
CCL27, and human CXCL12.

Chemokine has several unusual features, including a highly restricted expression pattern, because it is predominantly expressed in the skin (3, 4). Our initial experiments failed to detect binding of CCL27 to any of the known chemokine receptors, suggesting that it had a specific new receptor. However, there are several orphan “chemokine-like” receptors, including STRL33 (16) and GPR-15 (17) as well as GPR-2 (8). All of these are very likely to be chemokine receptors because they share a high degree of homology with known chemokine receptors, and they exhibit a characteristic DRY motif, found in other chemokine receptors. We tested CCL27 for binding to transfecteds of these receptors. Initially, we tested the published GPR-2 sequence, although it was missing part of the NH2 terminus. Transfectants of this molecule failed to show a calcium flux when tested against a panel of 35 human chemokines. We then sought to obtain a full-length clone encoding this receptor. Fig. 1 shows the sequence of a clone of human GPR-2, which was established from a cdNA library prepared from the 103 human B cell line. Comparison with the original published sequence (8) indicates that the first 8 aa were missing from the NH2 terminus of the original sequence (Fig. 1).

BAF/3 transfecteds of the full-length clone were tested against a panel of human chemokines using a calcium flux assay. Fig. 2, A and C show that both human and mouse CCL27 induced a specific calcium flux in the transfectants but not the parental BAF/3 cells, indicating that CCL27 binds GPR-2. Furthermore, CCL27 induced a strong chemotactic response in GPR-2 transfectants but not on the parental cells (Fig. 2B). Mouse CCL27 desensitized the response induced by human CCL27 and vice versa (Fig. 2C) demonstrating that they recognize the same receptor. BAF/3 cells are known to express CXCR4 endogenously, so we also tested the ligand of this receptor, CXCL12/SDF-1α, and observed the expected specific calcium flux in both the transfectants and the parental line. However, the latter response could not be desensitized by CCL27 in the transfectants, indicating that CXCL12 and CCL27 act through different chemokine receptors (Fig. 2C). Taken together, these results indicate that GPR-2 is a receptor for CCL27. Therefore, we have renamed this receptor CCR10, following the established chemokine receptor nomenclature. No other chemokine from a panel of 35 known human chemokines gave a response like CCL27 in the CCR10 transfectants (data not shown). Thus, CCR10 appears to be a specific CCL27 receptor. As mentioned above, transfecteds of the cdNA clone encoding the short version (lacking the first 8 aa; Fig. 1) of CCR10 did not respond to CCL27, like CCL27 in the transfectants, indicating that CXCL12 and CCL27 bind to different receptors (data not shown). Therefore, we have renamed this receptor CCR10, following the established chemokine receptor nomenclature. No other chemokine from a panel of 35 known human chemokines gave a response like CCL27 in the CCR10 transfectants (data not shown). Thus, CCR10 appears to be a specific CCL27 receptor. As mentioned above, transfecteds of the cdNA clone encoding the short version (lacking the first 8 aa; Fig. 1) of CCR10 did not respond to CCL27, suggesting that the amino terminus of CCR10 may be important for either receptor translation, stability, transportation to the cell surface, or direct ligand binding.

We then explored the expression of CCR10 in cdNA libraries derived from various human organs and cell types by real-time quantitative TaqMan PCR. Its expression is highest in the small intestine and colon, but is also expressed in fetal liver, fetal lung, fetal spleen, fetal testes, fetal brain, and uterus, among other tissues (data not shown). Moreover, analyses of cdNA libraries from various activated or resting human T cell clones suggest a complex regulation of this receptor (data not shown).

Given that secreted CCL27 is mainly expressed in skin, we investigated the expression of CCR10 and CCL27 in more detail in skin-related tissues including tissue samples from patients with psoriasis, atopic dermatitis, and cutaneous lupus erythematosus. To this end, we prepared cdNA from total RNA derived from these tissues that we then used for quantitative real-time RT-PCR analyses. We observed that CCL27 is constitutively and markedly expressed in normal or inflamed human skin (Fig. 3A). No significant difference in CCL27 expression using real-time quantitative PCR was observed between normal (n = 10) vs lesional skin from either psoriatic (n = 21), atopic dermatitis (n = 10), or lupus erythematosus patients (n = 10). We then analyzed the expression of hCCR10 in these samples (Fig. 3B) and observed uniformly low...
FIGURE 3. Quantitative TaqMan PCR analysis of CCL27 and CCR10 expression in normal vs inflamed skin as well as in cellular constituents of the skin. A and B, Pattern of CCL27 and CCR10 expression in normal skin and lesional skin of psoriatic patients, atopic dermatitis patients, and lupus erythematosus patients. Values are expressed as femtograms of target gene in 100 ng of total cDNA. Mean ± SD. C, CCL27 and CCR10 mRNA expression in cellular constituents of the skin. C, Analysis of CCL27 and CCR10 expression in cDNA obtained from cultured human primary keratinocytes, melanocytes, dermal fibroblasts, dermal microvascular endothelial cells, and epidermal γδ T cells (7-17) treated with medium alone or with TNF-α/IL-1β, TNF-α/IL-1β/IL-10, IFN-γ, or IL-4 for 6 or 18 h. D, Analysis of CCR10 expression in PBMCs, T cells, dendritic cells derived from CD34+ hematopoietic progenitor cells, or monocytes or LC. Values are expressed as femtograms of target gene in 100 ng of total cDNA. Representative data from single donors.
expression, which is not significantly up-regulated in any of the different inflammatory skin conditions. We also studied the expression of both CCL27 and CCR10 in various cellular components of the skin (Fig. 3C). CCL27 is exclusively expressed by human primary keratinocytes, although its expression is markedly up-regulated by IL-1β and TNF-α. Interestingly, IL-1β/TNF-α-induced CCL27 mRNA expression was down-regulated by additional IL-10 treatment (Fig. 3C). In contrast to CCL27, CCR10 is not expressed by keratinocytes (Fig. 3C) but is instead constitutively expressed in human primary melanocytes, dermal fibroblasts, and dermal microvascular endothelial cells. It also appears to be up-regulated in those cells upon induction with IL-1β and TNF-α (Fig. 3C). We also studied its expression levels in cDNA derived from PBMCs, T cells, and dendritic cells (Fig. 3D).

CCR10 is expressed in PBMCs, T cells, and LC, but not in either CD34+ hematopoietic progenitor-derived or monocyte-derived dendritic cells.

Discussion

In this study, we report the identification of a new chemokine receptor, which binds the recently described chemokine CTACK (4), also reported as ILC (3), ALP (5), and Eskine (6) but has now been renamed CCL27 according to a new systematic chemokine nomenclature (18). This new chemokine receptor was the previously orphan GPR-2 (8), which has now been renamed CCR10. Of all the human chemokines we tested, only CCL27 triggered a specific calcium flux and chemotactic responses in CCR10 transfectants, suggesting that CCR10 is a specific receptor for CCL27. The gene encoding CCR10 (GPR-2) has been previously mapped to human chromosome 17q21.1-2.13 (8). An important feature of the secreted chemokine form of CCL27 is that it is predominantly expressed in the skin (3, 4) by keratinocytes (4) (Fig. 3C). Interestingly, CCR10 expression was detected in skin as well as in several other tissues including small intestine, colon, brain, lung, liver, and testes (data not shown). CCL27 has at least two forms (6), but only one bears a signal peptide and is the form associated with predominant skin expression (3, 4). At this point, we do not know if the other intracellular forms of CCL27 bind CCR10 or if the latter receptor has any role in the physiology of the intracellular forms of CCL27. Given the predominant and selective expression of the secreted form of CCL27 in the skin (3, 4) by keratinocytes (Fig. 3C), it was surprising to find that CCR10 is expressed in various organs. This observation suggests that either CCL27 may be expressed in these tissues by a restricted cellular component under specific inflammatory or other pathogenic conditions, or there exists another ligand (which is either not a chemokine or not a known chemokine) for CCR10 that could be expressed in these tissues.

We focused our studies on the expression of CCR10 and CCL27 in human skin samples. In agreement with the discrete expression pattern of CCL27, we found CCR10 expressed in various normal cellular components of the skin including melanocytes, dermal fibroblasts, and dermal microvascular endothelial cells. CCL27 expression is induced in these cells by TNF-α and IL-1β, suggesting that CCL27 may play a role in the organization of cutaneous cellular components during inflammation. It is possible that CCL27/CCR10 may play a role in wound repair. Keratinocytes do not express CCR10, suggesting that CCL27/CCR10 represent a mechanism by which keratinocytes control the migration and/or differentiation of other cellular components of the skin in either normal or inflammatory conditions.

CCL27 is strongly expressed in both normal and inflamed skin. This suggests a homeostatic role as discussed above. CCR10 was also detected in both normal and diseased skin but at lower levels. This is consistent with other chemokine receptors, which, in contrast to chemokine ligands, do not represent abundant transcripts in the expressing cells. Lastly, we should consider the contribution of CCR10 expressed by T cells, because we first identified that CCL27 preferentially chemoattracts CLA+ T cells (4). Our data (Fig. 3) are consistent with the presence of CCR10 in a small population of peripheral blood T cells, as we predicted from our previous studies (4). Future experiments will aim at exploring its role in T cells. In conclusion, we have identified a novel chemokine receptor that is likely to be involved in both normal homeostasis as well as in inflammatory conditions of the skin.

Note Added in Proof. Another CC chemokine binding protein has been described in the literature under the aliases D6 or CCR10 (GenBank accession numbers Y12879, U94888, and U92803). However, a signaling function has not been shown for this molecule, and therefore the accepted criteria for a CCR designation have not been met. Accordingly, the Chemokine Receptor Nomenclature Committee has recommended that this molecule no longer be called CCR10 and instead that it be provisionally named D6. The Committee has recommended (P. Murphy, National Institutes of Health, Bethesda, MD, unpublished data) that the designation CCR9 be used for the TECK receptor (previous name GPR9-6; GenBank accession number AJ132337) and that CCR10 be used for the CCL27/CTACK receptor (previous name GPR-2).

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References


