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The Chemokine, CCL20, and Its Receptor, CCR6, in the Pathogenesis and Treatment of Psoriasis and Psoriatic Arthritis

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Abstract

Background: Chemokines represent a superfamily of immune-modulatory small protein molecules that regulate leukocyte migration to inflammatory sites through their chemoattractant and cell signaling properties. This review focuses on the immunological functions of the CCR6 chemokine receptor and is chemokine ligand, CCL20, that contribute to it role in inflammation in human psoriasis. **Methods:** Peer-reviewed relevant articles are searched and selected from 2000 to 2022 using the search engines including PubMed and Google Scholar. **Results:** After selectively reviewing and evaluating over seventy articles, a comprehensive overview on the immunology of CCL20-CCR6 axis in psoriasis and psoriatic arthritis, the X-ray crystal structures of CCL20 monomers, and the potential of developing clinical therapies targeting this axis is summarized. **Conclusions:** Over the past decade, preclinical studies carried out in animal models of psoriasis involving agents targeting CCL20-CCR6 axis have yielded promising results. Other studies that this axis may play a role in a number of other autoimmune diseases, including rheumatoid arthritis, suggesting a rationale for further investigation into this key signaling/migratory pathway.

Keywords

migration, chemokines, psoriasis, autoimmune disease, cell signaling

Introduction

Psoriasis is an autoimmune disease of the skin that up to 3% of a population, depending on the ethnic group.[1] Psoriasis can occur in several forms, the most common being plaque psoriasis which accounts for about 90% of cases.[2] Individuals with psoriasis are also at an increased risk of other co-morbidities, including psoriatic arthritis (PsA), metabolic syndrome or components of the syndrome, cardiovascular disorders, and several other diseases such as anxiety and depression, non-alcoholic fatty liver disease, Crohn's disease, and lymphoma.[3] Among psoriatic comorbidities, PsA is relatively common, affecting .06-.25% of the population in the United States and up to 30% of patients with psoriasis.[4] Biologic agents such as those targeting TNF-α, IL-17A, and IL-23 have made tremendous success in treating skin psoriasis with PASI 75 scores greater than 80% for the latest generation of biologics.[5-7] On the other hand, clinical response in PsA is not as impressive with less than 60% of patients with PsA achieving a clinically meaningful ACR20 response (a 20% improvement in joint symptoms and signs).[8-10] Therefore, improved strategies for diagnosing, preventing, and treating PsA is still an unmet need despite advances in psoriasis treatment.

Chemokines represent a superfamily of immune-modulatory small protein molecules that regulate leukocyte migration to inflammatory sites through their chemoattractant and cell signaling properties.[11] In 2012, we published a comprehensive review of the role of chemokines and their membrane receptors in psoriasis.[12] While updating new knowledge since 2012 would be daunting, we focus on a

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chemokine-chemokine receptor pair known as CCL20 and CCR6, respectively. CCL20, also known as MIP-3α or LARC, is an 8 kDa protein whose gene is mapped to 2q33-37.[13] At the tissue and organ level, CCL20 is expressed in peripheral tissues, including the skin, mucosal sites (lung and intestines), liver and thymus.[14] At a cellular level, it is expressed by keratinocytes,[15] lung and gut epithelial cells,[16] endothelial cells,[17] T cells,[18] macrophages[19] and neutrophils.[20] CCL20 is typically expressed at a low basal level, but can be strongly induced by proinflammatory signals, including primary cytokines (eg, TNF-α, IL-17A, IL-22),[15] injury[21] and Toll-like receptor (TLR) agonists originating from microbes.[22] The chemokine CCL20 is unusual in that it is one of the few chemokine ligands known to form a pair with only one G-protein-coupled receptor (GPCR): CXC receptor 6 (CCR6 or CD196). This exclusive binding is often referred to as the 'CCL20-CCR6 axis' in the literature, although studies suggest that CCR6 can bind to some β-defensins as well.[23] CCR6 is prominently expressed by IL-17A–producing helper T (Th17) cells,[24] regulatory T (Treg) cells,[25] memory T cells,[26] dendritic cells (DCs)[27] and most B-cell subsets.[28] Notably, CCR6 is expressed on virtually all human IL-17 A/F- and IL-22-producing CD4 + T cells, implicating CCR6 and CCL20 in IL-23/Th17/IL-22-mediated inflammation.[29] The role of CCR6 and CCL20 in Th17-related signaling will be detailed in Part II with a focus on psoriasis pathogenesis.

Notably, the CCR6-CCL20 axis has also been implicated in regulating the function of other types of immune cells. Recent studies suggest that CCR6-CCL20 interactions direct Treg to differentiate towards the pathogenic Th17 lineage by enhancing their expression of RORγt and IL-17A.[30,31] Immature DCs express CCR6 and respond to CCL20 in chemotaxis to sites of potential antigen entry.[32] CCR6 is also essential for appropriate anatomical positioning of memory B cells and the ability of memory B cells to be recalled to their cognate antigen, thus potentially contributing to humoral immunity.[33,34] The roles, however, of intrinsic CCR6-CCL20 signaling in DCs, B cells and innate lymphoid cell (ILCs) are largely unknown and, therefore, forms an active research area.

In this review, we will summarize the current research topics regarding the CCL20-CCR6 axis in psoriasis and PsA with particular emphasis of the advances that have been made over the last decade.

Part 1: The Structure and Function of CCR6 and CCL20

Recent work from multiple groups demonstrates that the highly selective coupling of CCL20 with CCR6 is a function of complementary structural features. All chemokines adopt a nearly invariant tertiary structure stabilized by two disulfide bonds. Chemokines form an extensive protein-protein interface with their GPCR partners. Docking of an unstructured and highly variable chemokine N-terminal sequence into the orthosteric pocket promotes specific chemokine-receptor contacts, stabilizes an active receptor conformation, and instigates intracellular signaling.[35]

Most chemokines have an N-terminal tail of 8 or more residues, with an average length of 10 residues (Figure 1A),[40] and alterations in the length or amino acid composition reduce or abrogate chemokine activity.[41] In contrast, the CCL20 N-terminus consists of only five amino acids (NH2-ASNFDCC) (Figure 1B). Despite its unusually short N-terminus, CCL20 binding to CCR6 expressed in HEK293 or COS-7 cells induced G protein and β-arrestin recruitment with EC_{50} values of ~1 nM,[42,43] reflecting an agonist potency comparable to other chemokine-receptor pairs.

In a detailed analysis of N-terminal structure-activity relationships, we discovered that, unlike most chemokines, CCL20 tolerates sequence extensions and truncations with minimal loss of activity.[44] Only one residue of the N-terminus, D5, was essential for CCL20 activity, and amino acid substitutions at that position resulted in substantial losses of binding affinity and agonist potency. Based on homology modeling of the CCL20-CCR6 interface, we predicted that this negatively charged residue of the chemokine forms a salt bridge with the positively charged side chain of R42 in the binding pocket of the receptor.[44]

It is generally understood that full activation of chemokine receptors to promote cellular chemotaxis results from binding of the monomeric chemokine ligand. The earliest chemokine structures, however, solved by NMR and X-ray crystallography revealed two distinct modes of dimerization corresponding to the CC and CXC subfamilies, and for a time there was uncertainty about the functional relevance of chemokine dimers. Proudfoot and colleagues resolved this question by demonstrating the necessity of chemokine dimerization for high affinity binding to glycosaminoglycans in the extracellular matrix.[45]

Key important functional differences exist between the two dimerization modes. CC-type dimers prevent receptor binding by sequestering residues of the N terminus within the dimer interface. In contrast, the N-terminus of a typical CXC dimer, which joins the β1 strands of opposing subunits, remains fully accessible and capable of receptor engagement. In 2008, the Volkman lab discovered that a CXCL12 molecule engineered to be exclusively dimeric bound its receptor CXCR4 with high affinity but lacked chemotactic activity.[46] Instead, the CXCL12 dimer acted as a G protein-biased agonist and functioned as potent inhibitor of metastatic cancer cell migration,[47] revealing the potential therapeutic utility of engineered chemokine dimers.

Structures of the CCL20 monomer were solved by NMR spectroscopy for the mouse[48] and human[49] proteins (Figure 1B), while X-ray crystal structures of human CCL20 unexpectedly revealed a CXC-type dimer[50,51] (Figure 1C). Based on speculation that dimeric CCL20 would be pharmacologically distinct from monomeric CCL20, Getschman et al. designed a CCL20 variant (S64 C) that forms

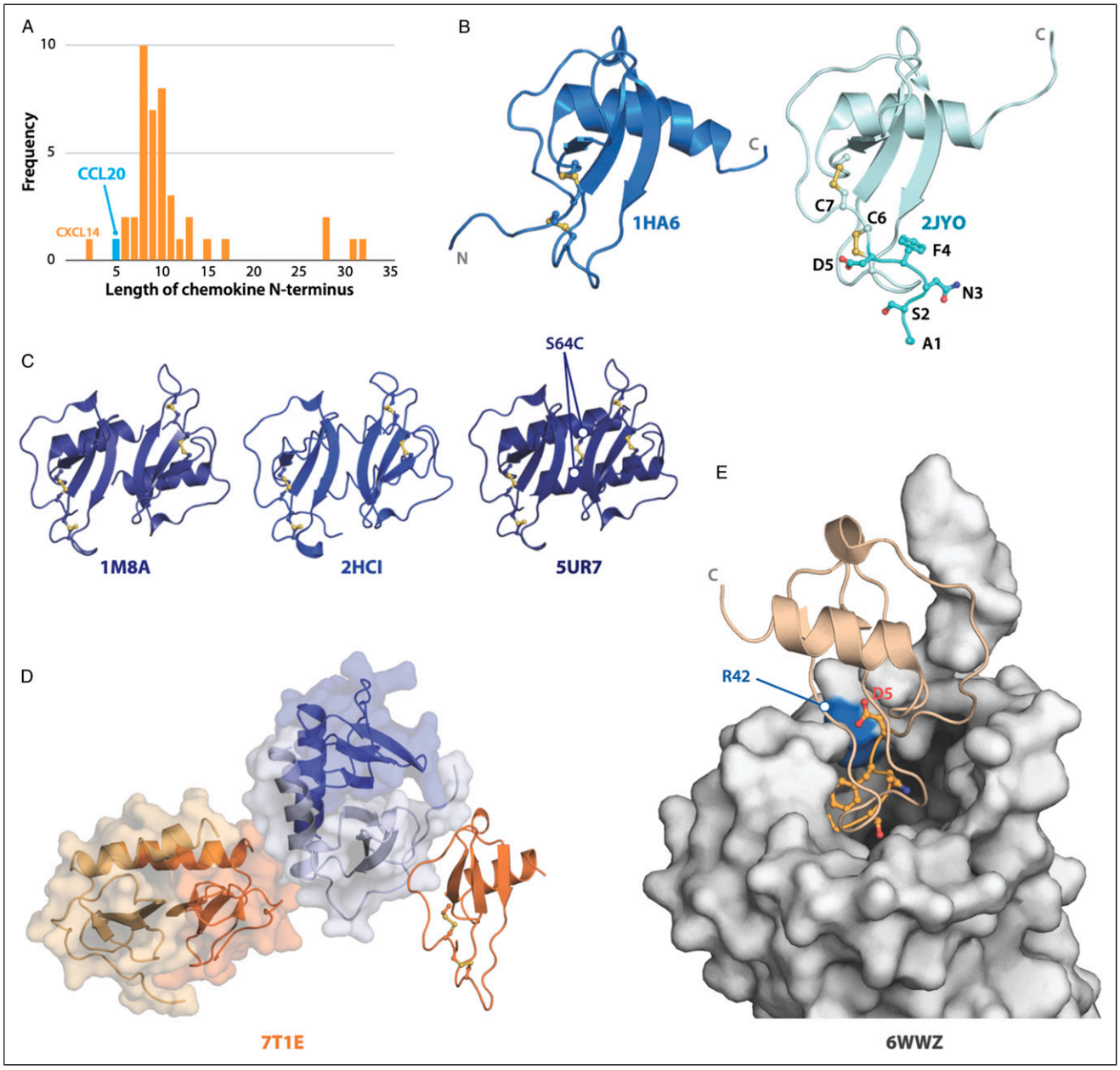

Figure 1. Structures of CCL20 and CCR6. A) Histogram of the length of flexible N-terminus for 47 human chemokines. Only orphan chemokine CXCL14 (3 residues) is shorter than CCL20 (5 residues). B) NMR structures of monomeric mouse and human CCL20. PDB IDs are shown and side chains of the N-terminus are labeled on the human CCL20 structure. C) Crystal structures of human CCL20 and the engineered CCL20 locked dimer. D) Crystal structure of human CCL20 at 1.46 Å resolution in a new space group (P 3_2 2 1) with five CCL20 molecules in the asymmetric unit. Initial crystallization conditions were identified by high-throughput screening (HTS) at the Hauptman-Woodward Medical Research Institute.[36] Optimization of crystallization conditions was conducted at 19°C by sitting drop vapor diffusion mixing equal volumes of protein and reservoir solution in 3 μL total volume. For optimization of conditions, reservoir solutions were prepared with .50 M succinic acid (pH 5.0, 5.2, 5.4, or 5.6) and PEG 3350 (13, 14, 15, 16, 17, or 18% *w*/*v*). The final reservoir solution of the crystal used for data collection contained .50 M succinic acid (pH 5.4) and 14% PEG 3350 (*w*/*v*). Diffraction data was processed using the autoProc toolbox[37] and the CCL20 structure was solved by molecular replacement. Geometry of the final structure was validated using Molprobity.[38,39] Data collection and refinement statistics for the final CCL20 model are listed in Table I and the coordinates for the structure deposited in the Protein Data Bank, PDB ID 7T1E. CCL20 dimers (gray) and the single CCL20 monomer (blue) correspond to one asymmetric unit. E) CryoEM structure of CCL20-CCR6 complex shows the interaction of D5 in the chemokine N-terminus with R42 in the TM1 helix of CCR6.

a disulfide-locked dimer and showed by X-ray crystallography that its structure is indistinguishable from the native CCL20 dimer (Figure 1B).[43] As described in Part 3, the CCL20 locked dimer inhibits CCR6-mediated chemotaxis and can serve as an anti-inflammatory agent with possible application in psoriatic diseases.

More recently, we solved the crystal structure of native, wild-type human CCL20 that contains two CXC-type dimers and a monomer in the asymmetric unit, providing the first high-resolution structure of a CCL20 monomer (Figure 1D and Table 1). The cryoEM structure of a CCL20-CCR6 complex[52] (Figure 1E) shows key D5 contacts that explain its functional importance originally described by Riutta et al[44] Additionally, D33, which is unusual at this position among CC chemokines (usually S or P; only CCL28 has a D at this position) forms a rare salt bridge with R286 of CCR6 (only CCR10 – a receptor for CCL28 – has an R at the same position).[36,37]

Collectively, the published structures and related structure-function data expose key factors in CCL20-CCR6 recognition

Table 1. Data collection and Refinement Statistics for CCL20.

	CCL20
Data collection[a]	
Source	APS LS-CAT-ID-G
Wavelength	.978560
Space group	P 3_2 2 1
Cell dimensions	
a, *b*, *c* (Å)	60.203, 60.203, 230.268
α, β, γ (°)	90, 90, 120
Resolution (Å)	77.09-1.46 (1.48-1.46)
No. of reflections	766 684
R_{merge}	.050 (.421)
R_{meas}	.053 (.493)
R_{pim}	.017 (.251)
CC(1/2)	.999 (.813)
$I/\sigma I$	24.0 (2.2)
Completeness (%)	98.7 (84.7)
Redundancy	9.0 (3.5)
Refinement[b]	
Resolution (Å)	34.61-1.46 (1.50-1.46)
No. of unique reflections	84 803
R_{cryst}/R_{free}	.163/0.173
No. atoms	3260
Protein	2760
Ion	100
Water	400
B-factors	
Protein	23.6
Ion	35.2
Water	34.3
R.m.s. deviations	
Bond lengths (Å)	.006
Bond angles (°)	.891
Ramachandran Stats	
Favored	98.45%
Allowed	1.55%

[a]Prior to data collection, crystals were flash frozen after passing through a cryoprotection solution of .5 M succinic acid (pH 5.4), 17% PEG 3350 (*w*/*v*), and 20% glycerol (*v*/*v*). X-ray diffraction data was gathered from a single crystal in one degree increments at 100 K on the LS-CAT ID-21-G beam line at the Advanced Photon Source (Argonne National Labs, Lemont, IL). Values in parentheses are for highest-resolution shell.

[b]A CCL20 X-ray structure (PDB ID 1M8A) devoid of water molecules was used as the search model. Phenix.AutoMR[87] solved initial phases and placed four CCL20 monomers in the asymmetric unit. A fifth CCL20 monomer plainly visible in the electron density was added manually. The model containing five CCL20 monomers in the asymmetric unit was then used as the search model and Phenix.AutoMR automatically built the majority of residues. The resulting model was completed through iterative rounds of manual model building in Coot[85] and refinement with Phenix.refine[84] using translational libration screw-motion (TLS) and individual atomic displacement parameters.

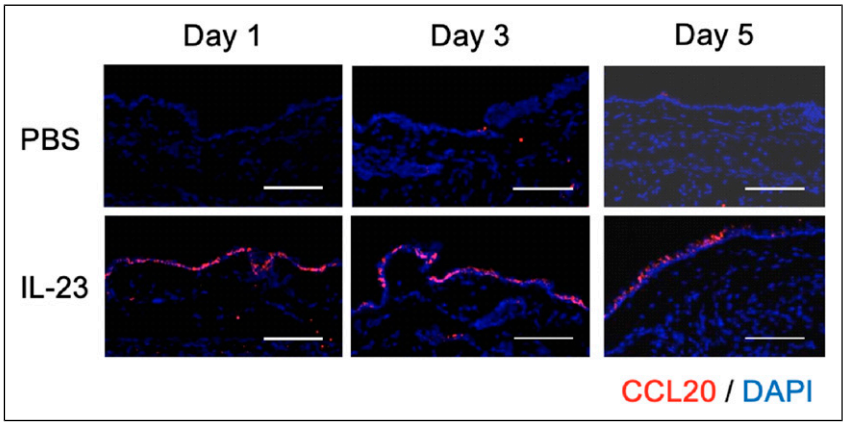

Figure 2. CCL20 expression in mouse epidermis is upregulated within 24 hr of IL23 injection. IL23 was injected into ears of WT mice at day 0, 2, and 4 as described.[62] Mice were euthanized on the indicated days and ears were stained with anti-CCL20 antibody (red) and DAPI (nuclear counterstain in blue). Isotype control staining showed no epidermal staining with either IL23 or PBS-injected ears (data not shown). Scale bar = 50 μm.

and signal transduction. Drug development efforts targeting the CCR6 orthosteric pocket will benefit from the recently published cryoEM structure of this complex.[52] The first 26 residues of the CCR6 N-terminus, however, are missing in the electron density. Structural studies of other complexes (eg CXCL12-CXCR4) show the receptor N-terminus wrapping around the chemokine to form an extensive protein-protein interface. There may be important CCL20-CCR6 contacts that were not captured by the cryoEM reconstruction, some of which may be specific to binding of the monomeric or dimeric form of the chemokine ligand. Understanding how CCL20 dimerization converts the pro-inflammatory chemokine into an anti-inflammatory CCR6 ligand deserves further structural investigation, particularly in light of promising results obtained for the CCL20 locked dimer in mouse models of psoriatic disease described below (Table 1).

Part 2: Immunology of CCL20-CCR6 Axis in Psoriasis and Psoriatic Arthritis

CCL20-CCR6 Axis in Psoriasis

In the pathogenesis of psoriasis, TNF-α/IL-23/IL-17 pathways play critical roles in generating the proinflammatory/hyperproliferative immune milieu that characterizes psoriatic lesions.[53] In the skin, the infiltrating dermal dendritic cells produce large amounts of IL-23 and induce the differentiation of IL-17A-producing T cells, such as Th17 cells, IL-17-producing γδ-low cells,[54] group 3 innate lymphoid cells (ILC3),[55,56] and other cells.[57] In combination with TNF and/or other proinflammatory cytokines, IL-17 stimulates keratinocytes to produce defensins and chemokines, which leads to the recruitment of additional inflammatory cells into the lesion.[53] CCR6 is associated with Th17 inflammatory processes[58] and is found prominently on Th17 cells,[24] IL-17-producing γδ-low cells,[59] and other cells in humans.[26,60] We and others have subsequently shown this receptor/chemokine pair to also play important roles in murine psoriasiform dermatitis (PsD). Notably, mice deficient in CCR6 fail to develop IL-23-induced, IL-22-dependent psoriasis-like inflammation.[61] Furthermore, CCR6 is pivotal in epidermal trafficking of IL-17/22-producing γδ-low cells.[54,62] CCL20 is typically expressed at a low basal level but can be strongly induced by proinflammatory signals, including primary cytokines.[63] We showed that keratinocytes express CCL20 early after IL-23 injection in a murine model (Figure 2).[62] Interestingly psoriasis exhibits induction of the disease at sites of trauma, often referred to as the Koebner or isomorphic phenomenon. We hypothesize that the early production of CCL20 from keratinocytes that is elicited by scratching may trigger psoriatic skin lesions via CCL20-CCR6 axis.[21]

Initiation of CCL20 and IL-17 signaling in keratinocyte is regulated by CARMA2 interaction with the ACT1-TRAF6 complex in an imiquimod-induced murine model.[64] Notably, CARMA2 is encoded by the *Card14* gene, which has been identified as a susceptibility gene in psoriasis.[65] It is now clear that IL-17-producing cells enhance the response of keratinocytes by creating an inflammatory loop based upon the IL-23/IL-17 axis.[57,66]

The involvement of CCR6 and CCL20 in psoriatic inflammation was first documented in humans by Homey et al[67] The CCL20 chemokine was upregulated in lesional psoriatic skin, and CCR6 expression was increased on circulating PBMCs from psoriasis patients compared to normal donors.[67] In human patients with psoriasis vulgaris, it has been reported that blood to skin recirculation of $CD4^+$ memory T cells is associated with cutaneous and systemic inflammation.[58] Specifically, circulating CCR6+ $CD4^+$ memory T cells showed positive correlation with serum C reactive protein (CRP) as a marker of systemic inflammation.[58] Moreover, skin-homing T cells often express the cutaneous lymphocyte-associated-antigen (CLA).[58,68] CLA is expressed mainly on

CCR6 + T cells and on CCR6+ CXCR3+ T cells but is also expressed on CCR6- CXCR3 + T cells.[58] Recently, a new population of $CD4^+$ T cells, peripheral helper T (Tph) cells, was identified.[69] Tph cells are divided into three groups based on their expression of CCR6 and CXCR3: CCR6- CXCR3 + Tph1 cells, CCR6- CXCR3- Tph2 cells, and CCR6 + CXCR3- Tph17 cells.[70] Another paper reported that the frequency and activation status of circulating CCR6 + CXCR3- Tph17 cells were elevated in patients with psoriasis vulgaris than in healthy controls and that the frequency positively correlated with disease severity.[71]

CCL20-CCR6 Axis in PsA

In contrast to psoriasis vulgaris, much less is known about the role of the CCL20-CCR6 axis in PsA because of the inaccessibility of affected human joints for routine biopsy and a lack of a murine model which reflects most of the features of human PsA. Hirota et al. reported that anti-CCL20 antibodies blocked development of murine joint injury in their rheumatoid arthritis-focused, collagen-induced arthritis model.[72] Our data, however, suggested that the role of CCR6 in the joint in experimental models of joint injury can vary, depending on the experimental setting. In C57/BL6 mice with PsD and concomitant joint injury through use of systemic IL-23 production using an IL-23 mini-circle DNA mode, CCR6-deficient mice had expected reduction of skin disease but joint disease, albeit, mild was unaffected.[73] In more autoimmune prone strains such as the B10.RIII strain in which both skin and joint involvement was substantially more severe than in C56/BL6 mice using the IL-23 mini-circle model, we were able to block both skin and joint inflammation in prevention and therapeutic settings using a novel CCR6 antagonist called CCL20 locked dimer (see Part III of this review).[74] Interestingly, the entheses of B10.RIII mice treated with IL-23 minicircle expressed high levels of CCL20, raising the possibility that the enthesitis that characterizes PsA may be driven in part by this chemokine.

Clinically, there is evidence that the CCL20-CCR6 axis may be involved in PsA in humans. In PsA patients, CCR6 expression was reported to be enriched on T cells[75] and ILC3[76] from synovial fluid (SF) of PsA patients. A high proportion of synovial IL-17A-expessing $CD8^+$ T cells in the SF of these patients also co-express CCR6. This expression of CCR6, however, is not restricted to the IL-17A + population.[75] An in vitro T cell migration assay revealed that $CD4^+$ and $CD8^+$ T cells migrating toward SF contained a lower percentage of CCR6 + cells and increased percentage of CXCR3 + cells.[66] A limited number of human PsA clinical studies further reported that expression of CCL20 in synovial tissue and CCL20 in SF strongly correlated with disease activity such as CRP levels,[77] and CCL20 was among 1 of 9 genes with concordant expression in SF cells and peripheral blood cells from PsA patients compared to osteoarthritis (OA) and psoriasis patients.[78] We recently reported that CCL20 (vs. more than 12 other chemokines) was expressed at higher levels in the joints of PsA patients compared to normal controls and patients with osteoarthritis.[74] Moreover, conditioned medium from cytokine-stimulated human tenocytes was able to stimulate chemotaxis of CCR6-expressing T cells.[74]

Part 3: CCL20-CCR6 Axis as a Therapeutic Target for Psoriasis and PsA

Targeting the CCR6/CCL20 Axis in Psoriasis

Over the past decade, preclinical studies carried out in animal models of psoriasis involving agents targeting CCL20-CCR6 axis have yielded promising results. In a mouse psoriasis model generated by intradermal IL-23 injection, local treatment with an anti-CCL20 antibody significantly reduced epidermal thickness, infiltration of CCR6+ γδ-low T cells and transcripts of IL-22 in the ear skin.[62] Humanized anti-CCR6 antibodies efficiently improved imiquimod (IMQ)-induced PsD in human CCR6-transgenic/mouse Ccr6-deficient mice.[79] Notably, the effect of anti-CCR6 antibodies was superior to that observed with the anti–IL-17 treatment at the same dose regime regarding the skin thickness and infiltration of IL-17A-producing T cells.

Recently, several small molecule antagonists of CCR6 have been developed and tested in preclinical studies. A potent and specific small molecule antagonist of the CCR6 chemokine receptor, CCX2553, was effective in reducing multiple aspects of psoriatic inflammation in two different murine models of the disease.[80] Subcutaneous administration of CCX2553 ameliorated skin inflammation in both the IL-23–induced ear swelling and the topical IMQ models, with significant reduction of the number of IL-17-secreting γδ T cells in inflamed skin. The anti-inflammatory effects of CCX2553 in the IMQ model were comparable to those of anti-IL-17RA treatment. Similarly, oral administration of CCX9664, a potent CCR6 antagonist, reduced skin inflammation in both psoriasis model generated by intradermal injection of IL-23 or topical application of IMQ.[81] Subcutaneous administration of CCX624, a dual CCR6 CXCR2 antagonist,[82] significantly alleviated IL-36α-induced murine psoriasiform inflammation accompanied by reduced accumulation of $CD4^+$ T cells, neutrophils, and inflammatory DCs. Its anti-psoriatic effects were better than those of an anti-IL-17RA monoclonal antibody (mAb) in reducing ear thickness and infiltration of CD4 T cells.

As a novel approach to blocking the CCL20-CCR6 axis, we engineered the monomeric wild-type CCL20 sequence with a single amino acid change such that the resulting expressed protein adopts a locked (via a disulfide bridge) dimeric structure that is nearly identical to spontaneous dimers of CCL20 that occur in nature at high

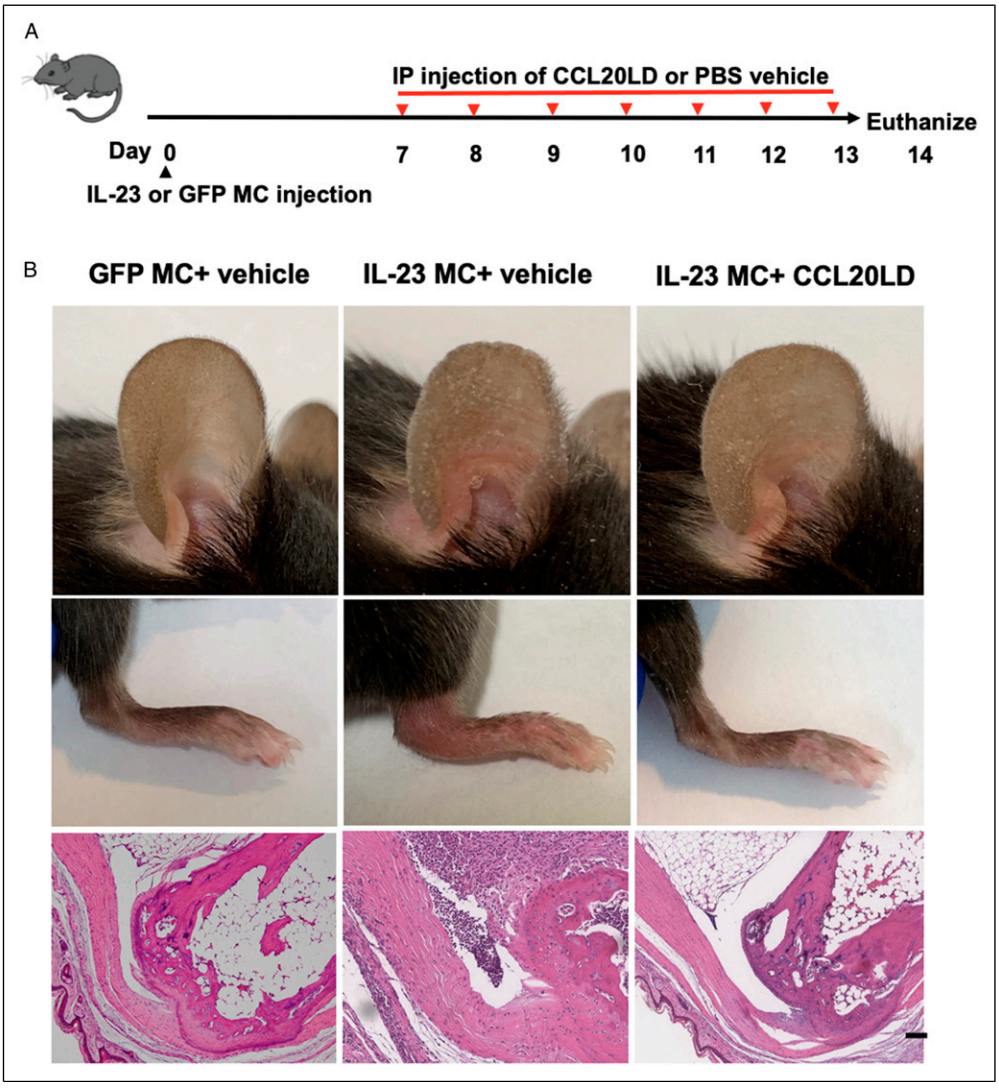

Figure 3. CCL20LD attenuates IL-23-mediated dermatitis, joint inflammation and enthesitis in a therapeutic manner. (A) Schematic illustration of experimental protocals.B10.RIII mice were treated with PBS vehicle or CCL20LD (100 μg) for 7 consecutive days beginning at day 7 after MC delivery. (B) Representative photographs of ear (upper panel), hind paws (middle panel) and H&E images showing enthesis (lower panel) at day 14. Scale bar = 100 μm.

concentrations.[43] The resulting dimeric molecule, called CCL20 locked dimer (CCL20LD), binds CCR6 with physiologic affinity but blocks the chemotactic activity of wild-type CCL20 and was able to ameliorate PsD in the IL-23 intradermal injection mouse model. Together, these preclinical data suggest the CCL20-CCR6 axis as a promising target for therapeutic intervention in human psoriasis.

In addition to psoriasis, emerging data also suggest that CCL20-CCR6 signaling is involved in the pathology of other inflammatory rheumatic diseases such as rheumatoid arthritis (RA).[83] Specific blockade, however, of CCL20 or CCR6 has only been evaluated so far in one murine model of RA using a mAb against CCR6.[72] In that investigation, $CD4^+$ T cells from SKG mice were transferred into syngeneic severe combined immunodeficiency mice and administered a blocking mAb against CCR6. Compared with a control mAb model, the mice had significantly reduced severity of arthritis, and migration of Th17 cells to the joint was impeded.

Targeting the CCR6/CCL20 Axis in PsA

In contrast to cutaneous psoriasis and RA, far less is known about the role of CCR6 in PsA due to the inaccessibility of affected human joints to routine biopsy and a lack of a murine model of PsA that fully reflects features of human PsA. Using an IL-23 minicircle DNA (MC)-based murine model with concurrent features of psoriasis-like skin and joint injuries,

we recently demonstrated that CCL20LD reduces not only IL-23-mediated skin inflammation but also attenuates joint inflammation.[74] When treated with preventative administration of CCL20LD, paw inflammation was significantly blocked as evidenced by a 60% reduction in arthritis incidence and a 95% reduction in severity. In a therapeutic model (Figure 3A), where treatment was started on day 7 following IL-23 MC injection (when animals had evidence of joint inflammation), mice receiving CCL20LD had substantially reduced clinical signs of skin and joint inflammation (Figure 3B), histologically less pannus formation and destruction of articular surfaces, and decreased levels of proinflammatory markers including IL-17, IL-1β and IL-6 at the molecular level.[74] The improvement of joint disease by CCL20LD was associated with a dampened inflammatory response in the entheses, an essential site of inflammation in PsA represented by the connective tissue linking tendons and ligaments with bones.[84] In the IL-23 MC model, we found that CD45 negative cells exhibited higher CCL20 gene expression and that healthy human tenocytes were able to produce CCL20 in response to activation with IL-1β. These results suggest that tenocytes are likely to be the major source of CCL20 in entheses.

Therapeutical targeting the CCR6-CCL20 axis may have a number of advantages. By specifically targeting this axis which is key in psoriatic inflammation, but not in other conditions such as atopic dermatitis, the higher specificity may result in less broad immunosuppression and, hence, decreased risk for infection. Second, despite the success of monoclonal antibodies that have been developed against TNF, IL-17A, and IL-23, all of these agents are subject to host anti-drug antibodies which can lead to diminished effectiveness and neutralization of these agents over time.[85] The nearly identical sequence of the locked CCL20 dimer compared to monomeric CCL20 may dramatically reduce the occurrence of anti-drug antibodies.

Despite much progress obtained in animal models, to our knowledge, no clinical trial results involving any CCL20-CCR6 blockade in relation to psoriasis have been published. GSK3050002, a humanized IgG1κ antibody with high binding affinity to human CCL20, was administered intravenously in a first-in-human study to evaluate its safety.[86] This agent was well tolerated in healthy male volunteers, and there were no deaths, serious adverse events or withdrawals due to adverse events. An experimental skin suction blister model in this study further showed that administration of GSK3050002 resulted in a dose-dependent reduction in the percentage of CCR6 + T-cells that were recruited into the skin blisters.

In summary, specific targeting of either the CCR6 receptor or the CCL20 chemokine ligand may be a useful strategy for treating psoriasis, PsA, RA, and, potentially, other Th17-mediated autoimmune diseases. Further clinical trials are required to determine its true therapeutic value in human patients.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: STH, BFV, and FCP have financial interest in and are officers of XLock Biosciences, which produces the CCL20LD.

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Consent

Patient consent was not necessary for the literature review.

Ethics

Ethics approval was not necessary for the literature review.

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References

1. Parisi R, Iskandar IYK, Kontopantelis E, et al. National, regional, and worldwide epidemiology of psoriasis: Systematic analysis and modelling study. *BMJ*. 2020;369:m1590.
2. Boehncke WH, Schon MP. Psoriasis. *Lancet*. 2015;386:983-994.
3. Oliveira Mde F, Rocha Bde O, Duarte GV. Psoriasis: Classical and emerging comorbidities. *An Bras Dermatol*. 2015;90:9-20.
4. Ogdie A, Weiss P. The epidemiology of psoriatic arthritis. *Rheum Dis Clin North Am*. 2015;41:545-568.
5. Langley RG, Elewski BE, Lebwohl M, et al. Secukinumab in plaque psoriasis–results of two phase 3 trials. *N Engl J Med*. 2014;371:326-338.
6. Griffiths CEM, Strober BE, van de Kerkhof P, et al. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. *N Engl J Med*. 2010;362:118-128.
7. Bilal J, Berlinberg A, Bhattacharjee S, Trost J, Riaz IB, Kurtzman DJB. A systematic review and meta-analysis of the efficacy and safety of the interleukin (IL)-12/23 and IL-17 inhibitors ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab and tildrakizumab for the treatment of moderate to severe plaque psoriasis. *J Dermatolog Treat*. 2018;29:569-578.
8. Deodhar A, Gottlieb AB, Boehncke WH, et al. Efficacy and safety of guselkumab in patients with active psoriatic arthritis: A randomised, double-blind, placebo-controlled, phase 2 study. *Lancet*. 2018;391:2213-2224.
9. McInnes IB, Kavanaugh A, Gottlieb AB, et al. Efficacy and safety of ustekinumab in patients with active psoriatic arthritis: 1

year results of the phase 3, multicentre, double-blind, placebo-controlled PSUMMIT 1 trial. *Lancet*. 2013;382:780-789.
10. McInnes IB, Mease PJ, Kirkham B, et al. Secukinumab, a human anti-interleukin-17A monoclonal antibody, in patients with psoriatic arthritis (FUTURE 2): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2015;386:1137-1146.
11. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006;354:610-621.
12. Mabuchi T, Chang TW, Quinter S, Hwang ST. Chemokine receptors in the pathogenesis and therapy of psoriasis. *J Dermatol Sci*. 2012;65:4-11.
13. Schutyser E, Struyf S, Van Damme J. The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev.* 2003;14:409-426.
14. Lee AYS, Eri R, Lyons AB, Grimm MC, Korner H. CC chemokine ligand 20 and its cognate receptor CCR6 in mucosal T cell immunology and inflammatory bowel disease: Odd couple or axis of evil? *Front Immunol*. 2013;4:194.
15. Harper EG, Guo C, Rizzo H, et al. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: Implications for psoriasis pathogenesis. *J Invest Dermatol*. 2009;129:2175-2183.
16. Reibman J, Hsu Y, Chen LC, Bleck B, Gordon T. Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol*. 2003;28:648-654.
17. Meissner A, Zilles O, Varona R, et al. CC chemokine ligand 20 partially controls adhesion of naive B cells to activated endothelial cells under shear stress. *Blood*. 2003;102:2724-2727.
18. Yamazaki T, Yang XO, Chung Y, et al. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol*. 2008;181:8391-8401.
19. Samaniego R, Gutierrez-Gonzalez A, Gutierrez-Seijo A, et al. CCL20 expression by tumor-associated macrophages predicts progression of human primary cutaneous melanoma. *Cancer Immunol Res*. 2018;6:267-275.
20. Scapini P, Laudanna C, Pinardi C, et al. Neutrophils produce biologically active macrophage inflammatory protein-3alpha (MIP-3alpha)/CCL20 and MIP-3beta/CCL19. *Eur J Immunol*. 2001;31:1981-1988.
21. Furue K, Ito T, Tanaka Y, et al. Cyto/chemokine profile of in vitro scratched keratinocyte model: Implications of significant upregulation of CCL20, CXCL8 and IL36G in Koebner phenomenon. *J Dermatol Sci.* 2019;94:244-251.
22. Sibartie S, O'Hara AM, Ryan J, et al. Modulation of pathogen-induced CCL20 secretion from HT-29 human intestinal epithelial cells by commensal bacteria. *BMC Immunol.* 2009;10:54.
23. Lee AYS, Phan TK, Hulett MD, Korner H. The relationship between CCR6 and its binding partners: Does the CCR6-CCL20 axis have to be extended? *Cytokine*. 2015;72:97-101.
24. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol.* 2008;180:214-221.
25. Kitamura K, Farber JM, Kelsall BL. CCR6 marks regulatory T cells as a colon-tropic, IL-10-producing phenotype. *J Immunol*. 2010;185:3295-3304.
26. Kondo T, Takata H, Takiguchi M. Functional expression of chemokine receptor CCR6 on human effector memory CD8+ T cells. *Eur J Immunol*. 2007;37:54-65.
27. Kim TG, Jee H, Fuentes-Duculan J, et al. Dermal clusters of mature dendritic cells and T cells are associated with the CCL20/CCR6 chemokine system in chronic psoriasis. *J Invest Dermatol*. 2014;134:1462-1465.
28. Lee AYS, Korner H. The CCR6-CCL20 axis in humoral immunity and T-B cell immunobiology. *Immunobiology.* 2019;224:449-454.
29. Hedrick MN, Lonsdorf AS, Hwang ST, Farber JM. CCR6 as a possible therapeutic target in psoriasis. *Expert Opin Ther Targets*. 2010;14:911-922.
30. Kulkarni N, Meitei HT, Sonar SA, et al. CCR6 signaling inhibits suppressor function of induced-Treg during gut inflammation. *J Autoimmun*. 2018;88:121-130.
31. Shen X, Zhang H, Xie H, et al. Reduced CCR6(+)IL-17A(+) Treg cells in blood and CCR6-dependent accumulation of IL-17A(+)Treg cells in lungs of patients with allergic asthma. *Front Immunol*. 2021;12:710750.
32. Ito T, Carson WFt., Cavassani KA, Connett JM, Kunkel SL. CCR6 as a mediator of immunity in the lung and gut. *Exp Cell Res*. 2011;317:613-619.
33. Elgueta R, Marks E, Nowak E, et al. CCR6-dependent positioning of memory B cells is essential for their ability to mount a recall response to antigen. *J Immunol*. 2015;194:505-513.
34. Suan D, Krautler NJ, Maag JLV, et al. CCR6 defines memory B cell precursors in mouse and human germinal centers, revealing light-zone location and predominant low antigen affinity. *Immunity.* 2017;47:1142-1153.e4.
35. Kleist AB, Getschman AE, Ziarek JJ, et al. New paradigms in chemokine receptor signal transduction: Moving beyond the two-site model. *Biochem Pharmacol*. 2016;114:53-68.
36. Luft JR, Collins RJ, Fehrman NA, Lauricella AM, Veatch CK, DeTitta GT. A deliberate approach to screening for initial crystallization conditions of biological macromolecules. *J Struct Biol*. 2003;142:170-179.
37. Vonrhein C, Flensburg C, Keller P, et al. Data processing and analysis with the autoPROC toolbox. *Acta Crystallogr D Biol Crystallogr.* 2011;67:293-302.
38. Chen VB, Arendall WB 3rd, Headd JJ, et al. MolProbity: All-atom structure validation for macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr.* 2010;66:12-21.
39. Davis IW, Leaver-Fay A, Chen VB, et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res*. 2007;35:W375-W383.
40. Wedemeyer MJ, Mahn SA, Getschman AE, et al. The chemokine X-factor: Structure-function analysis of the CXC motif at CXCR4 and ACKR3. *J Biol Chem*. 2020;295:13927-13939.
41. Chevigne A, Fievez V, Schmit JC, Deroo S. Engineering and screening the N-terminus of chemokines for drug discovery. *Biochem Pharmacol*. 2011;82:1438-1456.

42. Julian B, Gao K, Harwood BN, Beinborn M, Kopin AS. Mutation-induced functional alterations of CCR6. *J Pharmacol Exp Ther*. 2017;360:106-116.
43. Getschman AE, Imai Y, Larsen O, et al. Protein engineering of the chemokine CCL20 prevents psoriasiform dermatitis in an IL-23-dependent murine model. *Proc Natl Acad Sci U S A*. 2017; 114:12460-12465.
44. Riutta SJ, Larsen O, Getschman AE, Rosenkilde MM, Hwang ST, Volkman BF. Mutational analysis of CCL20 reveals flexibility of N-terminal amino acid composition and length. *J Leukoc Biol*. 2018;104:423-434.
45. Proudfoot AEI, Handel TM, Johnson Z, et al. Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proc Natl Acad Sci U S A*. 2003; 100:1885-1890.
46. Veldkamp CT, Seibert C, Peterson FC, et al. Structural basis of CXCR4 sulfotyrosine recognition by the chemokine SDF-1/ CXCL12. *Sci Signal*. 2008;1:ra4.
47. Drury LJ, Ziarek JJ, Gravel S, et al. Monomeric and dimeric CXCL12 inhibit metastasis through distinct CXCR4 interactions and signaling pathways. *Proc Natl Acad Sci U S A*. 2011;108: 17655-17660.
48. Perez-Canadillas JM, Zaballos A, et al. NMR solution structure of murine CCL20/MIP-3alpha, a chemokine that specifically chemoattracts immature dendritic cells and lymphocytes through its highly specific interaction with the beta-chemokine receptor CCR6. *J Biol Chem*. 2001;276:28372-28379.
49. Chan DI, Hunter HN, Tack BF, Vogel HJ. Human macrophage inflammatory protein 3alpha: Protein and peptide nuclear magnetic resonance solution structures, dimerization, dynamics, and anti-infective properties. *Antimicrob Agents Chemother*. 2008;52:883-894.
50. Hoover DM, Boulegue C, Yang D, et al. The structure of human macrophage inflammatory protein-3alpha/CCL20. Linking antimicrobial and CC chemokine receptor-6-binding activities with human beta-defensins. *J Biol Chem*. 2002;277:37647-37654.
51. Malik ZA, Tack BF. Structure of human MIP-3alpha chemokine. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2006;62: 631-634.
52. Wasilko DJ, Johnson ZL, Ammirati M, et al. Structural basis for chemokine receptor CCR6 activation by the endogenous protein ligand CCL20. *Nat Commun*. 2020;11:3031.
53. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol*. 2014;32:227-255.
54. Mabuchi T, Takekoshi T, Hwang ST. Epidermal CCR6+ γδ T cells are major producers of IL-22 and IL-17 in a murine model of psoriasiform dermatitis. *J Immunol*. 2011;187:5026-5031.
55. Villanova F, Flutter B, Tosi I, et al. Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J Invest Dermatol*. 2014;134: 984-991.
56. Dyring-Andersen B, Geisler C, Agerbeck C, et al. Increased number and frequency of group 3 innate lymphoid cells in nonlesional psoriatic skin. *Br J Dermatol*. 2014;170:609-616.
57. Lynde CW, Poulin Y, Vender R, Bourcier M, Khalil S. Interleukin 17A: Toward a new understanding of psoriasis pathogenesis. *J Am Acad Dermatol*. 2014;71:141-150.
58. Diani M, Galasso M, Cozzi C, et al. Blood to skin recirculation of CD4(+) memory T cells associates with cutaneous and systemic manifestations of psoriatic disease. *Clin Immunol*. 2017;180:84-94.
59. Laggner U, Di Meglio P, Perera GK, et al. Identification of a novel proinflammatory human skin-homing Vγ9Vδ2 T cell subset with a potential role in psoriasis. *J Immunol*. 2011;187: 2783-2793.
60. Kitamura K, Farber JM, Kelsall BL. CCR6 marks regulatory T cells as a colon-tropic, IL-10-producing phenotype. *J Immunol*. 2010;185:3295-3304.
61. Hedrick MN, Lonsdorf AS, Shirakawa AK, et al. CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. *J Clin Invest*. 2009;119:2317-2329.
62. Mabuchi T, Singh TP, Takekoshi T, et al. CCR6 is required for epidermal trafficking of γδ-T cells in an IL-23-induced model of psoriasiform dermatitis. *J Invest Dermatol*. 2013;133:164-171.
63. Harper EG, Guo C, Rizzo H, et al. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: Implications for psoriasis pathogenesis. *J Invest Dermatol*. 2009;129:2175-2183.
64. Wang M, Zhang S, Zheng G, et al. Gain-of-function mutation of Card14 leads to spontaneous psoriasis-like skin inflammation through enhanced keratinocyte response to IL-17A. *Immunity*. 2018;49:66-79.e5.
65. Jordan CT, Cao L, Roberson ED, et al. PSORS2 is due to mutations in CARD14. *Am J Hum Genet*. 2012;90:784-795.
66. Diani M, Casciano F, Marongiu L, et al. Increased frequency of activated CD8(+) T cell effectors in patients with psoriatic arthritis. *Sci Rep*. 2019;9:10870.
67. Homey B, Dieu-Nosjean MC, Wiesenborn A, et al. Up-regulation of macrophage inflammatory protein-3 alpha/ CCL20 and CC chemokine receptor 6 in psoriasis. *J Immunol*. 2000;164:6621-6632.
68. Davison SC, Ballsdon A, Allen MH, Barker JN. Early migration of cutaneous lymphocyte-associated antigen (CLA) positive T cells into evolving psoriatic plaques. *Exp Dermatol*. 2001;10: 280-285.
69. Rao DA, Gurish MF, Marshall JL, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature*. 2017;542:110-114.
70. Lin J, Yu Y, Ma J, Ren C, Chen W. PD-1+CXCR5-CD4+T cells are correlated with the severity of systemic lupus erythematosus. *Rheumatology (Oxford)*. 2019;58:2188-2192.
71. Liu W, Zhou X, Wang A, Ma J, Bai Y. Increased peripheral helper T cells type 17 subset correlates with the severity of psoriasis vulgaris. *Immunol Lett*. 2021;229:48-54.
72. Hirota K, Yoshitomi H, Hashimoto M, et al. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med*. 2007;204:2803-2812.

73. Shi Z, Garcia-Melchor E, Wu X, et al. Differential requirement for CCR6 in IL-23-mediated skin and joint inflammation. *J Invest Dermatol*. 2020;140:2386-2397.
74. Shi Z, Garcia-Melchor E, Wu X, et al. Targeting the CCR6/CCL20 axis in entheseal and cutaneous inflammation. *Arthritis Rheumatol*. 2021;73:2271-2281.
75. Steel KJA, Srenathan U, Ridley M, et al. Polyfunctional, proinflammatory, tissue-resident memory phenotype and function of synovial interleukin-17A+CD8+ T cells in psoriatic arthritis. *Arthritis Rheumatol*. 2020;72:435-447.
76. Leijten EF, van Kempen TS, Boes M, et al. Brief report: Enrichment of activated group 3 innate lymphoid cells in psoriatic arthritis synovial fluid. *Arthritis Rheumatol*. 2015;67:2673-2678.
77. Celis R, Planell N, Fernandez-Sueiro JL, et al. Synovial cytokine expression in psoriatic arthritis and associations with lymphoid neogenesis and clinical features. *Arthritis Res Ther*. 2012;14:R93.
78. Abji F, Pollock RA, Liang K, Chandran V, Gladman DD. Th17 gene expression in psoriatic arthritis synovial fluid and peripheral blood compared to osteoarthritis and cutaneous psoriasis. *Clin Exp Rheumatol*. 2018;36:486-489.
79. Robert R, Ang C, Sun G, et al. Essential role for CCR6 in certain inflammatory diseases demonstrated using specific antagonist and knockin mice. *JCI Insight*. 2017;2:e94821.
80. Campbell JJ, Ebsworth K, Ertl LS, et al. IL-17-secreting γδ T cells are completely dependent upon CCR6 for homing to inflamed skin. *J Immunol*. 2017;199:3129-3136.
81. Daniel Dairaghi PZ, Leleti M, Berahovich R, et al. *Inhibition of Chemokine Receptors CCR1 and CCR6 as Promising Therapies for Autoimmune Diseases Such as Rheumatoid Arthritis and Psoriasis*. ACR/ARHP Annual Meeting; 2013, Abstract No 1792.
82. Campbell JJ, Ebsworth K, Ertl LS, et al. Efficacy of chemokine receptor inhibition in treating IL-36α-induced psoriasiform inflammation. *J Immunol*. 2019;202:1687-1692.
83. Lee AYS, Korner H. CCR6 and CCL20: Emerging players in the pathogenesis of rheumatoid arthritis. *Immunol Cell Biol*. 2014;92:354-358.
84. Kaeley GS, Eder L, Aydin SZ, Gutierrez M, Bakewell C. Enthesitis: A hallmark of psoriatic arthritis. *Semin Arthritis Rheum*. 2018;48:35-43.
85. Hsu L, Armstrong AW. Anti-drug antibodies in psoriasis: A critical evaluation of clinical significance and impact on treatment response. *Expert Rev Clin Immunol*. 2013;9:949-958.
86. Bouma G, Zamuner S, Hicks K, et al. CCL20 neutralization by a monoclonal antibody in healthy subjects selectively inhibits recruitment of CCR6(+) cells in an experimental suction blister. *Br J Clin Pharmacol*. 2017;83:1976-1990.
87. Adams PD, Afonine PV, Bunkoczi G, et al. PHENIX: A comprehensive python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr*. 2010;66:213-221.