CCL17 Promotes Intestinal Inflammation in Mice and Counteracts Regulatory T Cell–Mediated Protection From Colitis

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BACKGROUND & AIMS: Priming of T cells by dendritic cells (DCs) in the intestinal mucosa and associated lymphoid tissues helps maintain mucosal tolerance but also contributes to the development of chronic intestinal inflammation. Chemokines regulate the intestinal immune response and can contribute to pathogenesis of inflammatory bowel diseases. We investigated the role of the chemokine CCL17, which is expressed by conventional DCs in the intestine and is up-regulated during colitis. **METHODS:** Colitis was induced by administration of dextran sodium sulfate (DSS) to mice or transfer of T cells to lymphopenic mice. Colitis activity was monitored by body weight assessment, histologic scoring, and cytokine profile analysis. The direct effects of CCL17 on DCs and the indirect effects on differentiation of T helper (Th) cells were determined in vitro and ex vivo. **RESULTS:** Mice that lacked CCL17 (*Ccl17*$^{E/E}$ mice) were protected from induction of severe colitis by DSS or T-cell transfer. Colonic mucosa and mesenteric lymph nodes from *Ccl17*-deficient mice produced lower levels of proinflammatory cytokines. The population of Foxp3$^+$ regulatory T cells (Tregs) was expanded in *Ccl17*$^{E/E}$ mice and required for long-term protection from colitis. CCR4 expression by transferred T cells was not required for induction of colitis, but CCR4 expression by the recipients was required. CCL17 promoted Toll-like receptor–induced secretion of interleukin-12 and interleukin-23 by DCs in an autocrine manner, promoted differentiation of Th1 and Th17 cells, and reduced induction of Foxp3$^+$ Treg cells. **CONCLUSIONS: The chemokine CCL17 is required for induction of intestinal inflammation in mice. CCL17 has an autocrine effect on DCs that promotes production of inflammatory cytokines and activation of Th1 and Th17 cells and reduces expansion of Treg cells.**

Keywords: TLR; IBD; Crohn's Disease; Mouse Model.

Inflammatory bowel disease (IBD) is characterized by a failure to regulate the immune response against luminal antigens, leading to a chronic relapsing inflammation of the gastrointestinal tract. This dysregulated immune response is sustained by the influx of naïve T cells that have not adapted to the mucosal milieu and are now exposed to luminal antigens.[1] The differentiation of naïve T cells recruited into the mucosa requires their interaction with antigen-presenting cells. This interaction is particularly crucial for the differentiation of CD4$^+$ T cells to proinflammatory T-helper (Th) 1 cells, interleukin (IL)-17–producing Th cells (Th17), and forkhead box P3 (Foxp3)$^+$ regulatory T cells (Tregs).[2] Dendritic cells (DCs) are professional antigen-presenting cells that attract appropriate T-cell populations by producing chemokines and shape the differentiation of these T cells via the production of cytokines.

The chemokine CCL (chemokine [C-C motif] ligand) 17, which is expressed by CD11b$^+$ DCs outside the spleen and binds to CCR4 (chemokine [C-C motif] receptor 4), is essential for the homing of CD4$^+$ T cells to the skin[3,4] and the lung.[5]

CCR4, the only known receptor for CCL17 and also for CCL22, is not only expressed by CD4$^+$ T cells, but also by CD8$^+$ cytotoxic T lymphocytes, natural killer cells, macrophages, and subsets of DCs.[6–9] On CD4$^+$ T cells, CCR4 is expressed on Th17 and Th2 effector cells as well as on Tregs. *Ccr4*-deficient Tregs do not migrate to mesenteric lymph nodes (MLNs) and fail to suppress colitis in the T-cell transfer colitis model.[10] Studies in *Ccr4*- and *Ccl17*-deficient mice identified many different and partly conflicting functions of this molecule. *Ccr4*-deficient mice fail to develop allograft tolerance due to diminished CCR4- and CCL22-dependent recruitment of Tregs to the cardiac allograft.[11] In contrast, cardiac allograft survival is prolonged in *Ccl17*-deficient mice.[12] In a murine model of atopic dermatitis, the absence of CCL17, but not the absence of CCR4, reduces inflammation and the migration of cutaneous DCs to lymph nodes.[13] These examples show the nonredundant function of the chemokine CCL17, which is not overlapping with that of CCR4.

BASIC AND TRANSLATIONAL AT

Abbreviations used in this paper: BMDC, bone marrow–derived dendritic cell; DC, dendritic cell; DSS, dextran sodium sulfate; eGFP, enhanced green fluorescent protein; IFN, interferon; IL, interleukin; LPL, lamina propria leukocyte; LPS, lipopolysaccharide; IEL, intraepithelial leukocyte; MLN, mesenteric lymph node; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; TGF, transforming growth factor; Th, T helper; TLR, Toll-like receptor; Treg, regulatory T cell; WT, wild-type.

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Little is known concerning the role of CCL17 in T-cell homing to the intestinal mucosa. CCL17 expression is up-regulated in the inflamed mucosa during experimental mouse models of colitis,[14,15] and one report found that CCL17 expression is up-regulated in the mucosa of patients with Crohn's disease.[16] However, it is not known to what extent and by which mechanism CCL17 influences the course of colitis.

We show that *Ccl17*-deficient mice are protected from colitis induced by naïve $CD4^+$ T-cell transfer and that this protection is independent of CCR4 expression on the transferred T cells. In the absence of CCL17, proinflammatory cytokine production by DCs and effector T cells is significantly reduced and Tregs are expanded, conferring long-term protection against colitis.

Materials and Methods

Mice

Mice were on the C57BL/6 background (at least 10 generations backcrossed). *Rag* (recombination-activating gene)$1^{-/-}$, *Ccl17*$^{eGFP/eGFP}$ (*Ccl17*$^{E/E}$),[12] *Rag1*$^{-/-}$*Ccl17*$^{E/E}$, *Ccr4*$^{-/-}$, *Rag1*$^{-/-}$*Ccr4*$^{-/-}$, OTII, and DEREG 23.2 mice[17] were bred in our specific pathogen-free animal facility. Experiments were performed in accordance with the German animal care and ethics legislation and approved by the local government authorities.

See Supplementary Materials and Methods for information on media and reagents, bone marrow–derived dendritic cells (BMDCs), isolation of colonic lamina propria leukocytes (LPLs) and intraepithelial leukocytes (IELs), flow cytometry, enzyme-linked immunosorbent assay, RNA isolation, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Isolation of T Cells and DCs

T cells (97% $CD45RB^{hi}$) were isolated using the $CD4^+CD62L^+$ T Cell Isolation Kit II (Miltenyi Biotec, Bergisch-Gladbach, Germany). DCs were isolated from MLNs after digestion for 30 minutes at 37°C using CD11c-MicroBeads (Miltenyi Biotec).

In Vitro Stimulation and Coculture

BMDCs were stimulated with lipopolysaccharide (LPS) (Sigma-Aldrich, Munich, Germany), Pam_3Cys-SKKKK (Pam_3Cys) (EMC microcollections GmbH, Tübingen, Germany), and/or recombinant murine CCL17 (R&D Systems, Minneapolis, MN) at 1×10^6 cells/mL. Cells were harvested for messenger RNA (mRNA) isolation after 6 hours. Supernatants were collected for enzyme-linked immunosorbent assay after 24 hours. DCs were pulsed with 5 μg/mL OVA-peptide (amino acids 323–339, ISQAVHAAHAEINEAGR; GenScript, Piscataway, NJ) and were cocultured 1:10 with $CD4^+CD62L^+$ OTII T cells labeled with carboxyfluorescein succinimidyl ester (1 μmol/L) for 4 days in the presence of 100 ng/mL LPS.

Induction of Colitis

Dextran sodium sulfate (DSS) colitis was induced by adding 4% (wt/vol) DSS to the drinking water for 5 days. T-cell transfer colitis was induced by adoptive transfer of 3×10^5 $CD4^+CD62L^+$ splenic T cells intraperitoneally into *Rag1*$^{-/-}$ mice. Mice were killed when they had lost 20% of their initial body weight.

Histology

Histologic scoring was performed as described.[18]

Statistical Analysis

Data are shown as mean ± SD. Student *t* test or one-way analysis of variance followed by Dunn's test or Student–Newman–Keuls test were performed using SigmaStat (Systat Software GmbH, Erkrath, Germany). *P* values less than .05 were considered to indicate statistically significant differences ($^*P < .05$, $^{**}P < .01$, $^{***}P < .001$).

Results

Ccl17*-Deficient Mice Are Protected From Severe Experimental Colitis*

Ccl17 mRNA expression in the colon is induced during active colitis.[15] We investigated the expression of CCL17 in DCs during experimental murine colitis using *Ccl17*$^{E/E}$ reporter mice expressing enhanced green fluorescent protein (eGFP) instead of CCL17. DSS treatment led to an increase in the percentage of *Ccl17*-eGFP–expressing DCs in the MLNs of *Ccl17*$^{E/E}$ mice (Figure 1*A*). Also, a 2- to 3-fold increase in the percentage of *Ccl17*-eGFP$^+$ DCs was found in MLNs, LPLs, and IELs of *Rag1*$^{-/-}$*Ccl17*$^{E/E}$ mice, which had received a $CD4^+CD62L^+$ T-cell transfer for induction of colitis 21 days earlier compared with control mice. No induction of CCL17 expression was found before the onset of colitis (Figure 1*B*). eGFP$^+$ cells expressed high levels of CD11c, CD11b, and major histocompatibility complex class II but very low levels of F4/80 (Supplementary Figure 1).

In the DSS model as well as the T-cell transfer model, severe colitis did not develop in the absence of CCL17. The induction of colitis by DSS led to a significantly reduced weight loss in *Ccl17*$^{E/E}$ animals (−6.2% ± 2.7 %) compared with wild-type animals (−15.9% ± 4.3%) at day 7 (Figure 1*C*, *right panel*). *Ccl17*$^{E/E}$ mice had significantly less colonic shortening and a lower histologic score (2.1 ± 2.1 [*Ccl17*$^{E/E}$] vs 4.1 ± 2.0 [wild-type]), representing diminished colonic inflammation (Figure 1*D*). The induction of colitis by T-cell transfer also led to a significantly blunted reduction in body weight in *Rag1*$^{-/-}$*Ccl17*$^{E/E}$ compared with *Rag1*$^{-/-}$ control mice (+0.1% ± 6.3% vs −18.9% ± 8.1%, day 21) (Figure 1*E*, *right panel*), and colonic inflammation was also almost entirely suppressed in *Rag1*$^{-/-}$*Ccl17*$^{E/E}$ mice (histologic score 0.3 ± 0.4 vs 3.5 ± 1.5, day 21) (Figure 1*F*). These findings show that CCL17 plays a crucial role in development of colitis and that its absence protects from active colitis.

Lower Induction of Proinflammatory Cytokines During Colitis in Ccl17$^{E/E}$ Mice

To gain further insight into the proinflammatory activity of CCL17, we investigated the cytokine expression

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