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*J Immunol* 2008; 181:6536-6545; doi: 10.4049/jimmunol.181.9.6536

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Differential Regulation of Chemokines by IL-17 in Colonic Epithelial Cells

Jimmy W. Lee,* Ping Wang,† Michael G. Kattah,‡ Sawsan Youssef,‡ Lawrence Steinman,†‡ Kathryn DeFea,2* and Daniel S. Straus2*

The IL-23/IL-17 pathway plays an important role in chronic inflammatory diseases, including inflammatory bowel disease. Inflammatory bowel disease, intestinal epithelial cells are an important source of chemokines that recruit inflammatory cells. We examined the effect of IL-17 on chemokine expression of HT-29 colonic epithelial cells. IL-17 strongly repressed TNF-α-stimulated expression of CXCL10, CXCL11, and CCL5, but synergized with TNF-α for induction of CXCL8, CXCL1, and CCL20 mRNAs. For CXCL10, IL-17 strongly inhibited promoter activity but had no effect on mRNA stability. In contrast, for CXCL8, IL-17 slightly decreased promoter activity but stabilized its normally unstable mRNA, leading to a net increase in steady-state mRNA abundance. IL-17 synergized with TNF-α in transactivating the epidermal growth factor receptor (EGFR) and in activating ERK and p38 MAPK. The p38 and ERK pathway inhibitors SB203580 and U0126 reversed the repressive effect of IL-17 on CXCL10 mRNA abundance and promoter activity and also reversed the inductive effect of IL-17 on CXCL8 mRNA, indicating that MAPK signaling mediates both the transcriptional repression of CXCL10 and the stabilization of CXCL8 mRNA by IL-17. The EGFR kinase inhibitor AG1478 partially reversed the effects of IL-17 on CXCL8 and CXCL10 mRNA, demonstrating a role for EGFR in downstream IL-17 signaling. The overall results indicate a positive effect of IL-17 on chemokines that recruit neutrophils (CXCL8 and CXCL1), and Th17 cells (CCL20). In contrast, IL-17 represses expression of CXCL10, CXCL11, and CCR5, three chemokines that selectively recruit Th1 but not other effector T cells. The Journal of Immunology, 2008, 181: 6536–6545.

Crohn’s disease is an inflammatory bowel disease with a chronic relapsing and remitting course (1). The immunopathology in Crohn’s disease was initially characterized as Th1 polarized, with high levels of the Th1 cytokine IFN-γ and low levels of the Th2 cytokines IL-4, IL-5, and IL-13 present in inflamed tissue (2). More recently, a newly defined class of IL-17-producing CD4+ T cells, termed Th17, has been found to play a key role in inflammatory and autoimmune diseases, including Crohn’s disease (3–5). Human Th17 cells are characterized by expression of the transcription factor RORγt, the IL-23 receptor, and the chemokine receptor CCR6 (4, 6–9). Conditions that favor Th1 differentiation inhibit differentiation of Th17 cells. In particular, the Th1 cytokine IFN-γ antagonizes the differentiation of naïve mouse CD4+ T cells into Th17 cells (10, 11) and the induction of Th17 cells in human CD4+ CD45RO+ memory T cell populations (12). Moreover, IFN-γ inhibits production of the CCR6 ligand CCL20, a chemokine that selectively recruits Th17 cells (9). Despite the negative effect of IFN-γ on Th17 differentiation and recruitment, the exact relationship between Th1 and Th17 cells remains poorly understood. Although Th1-polarizing conditions inhibit induction of Th17 cells, in some contexts the two cell types seem to cooperate in initiating and/or perpetuating chronic inflammation. For example, in human inflammatory diseases including Crohn’s disease (4) and psoriasis (6) and in the IL-10 knockout mouse model of inflammatory bowel disease (13), high levels of IFN-γ and IL-17 may be found together in the same specimens of inflamed tissue. Further complicating the understanding of Th1-Th17 interaction, a newly identified subset of T cells termed Th17 has been found to have features of both Th1 and Th17, including production of both IFN-γ and IL17 (4, 7, 12, 14). Finally, in addition to being produced by Th17 cells, IL-17 is also produced by cells that participate in the innate immune response, including monocytes, granulocytes, NK1.1− invariant NKT cells, and γδ T cells (15–18).

Chemokines play a central role in recruiting immune cells to the site of inflammation, and the role of IL-17 in regulating chemokine production is complex, with positive effects exerted on the production of some chemokines and negative effects exerted on others. IL-17 synergizes with TNF-α in up-regulating the expression of a subset of chemokines and cytokines including CXCL1, CXCL8, CCL20, and IL-6 (19–22). Whereas TNF-α induces transcription of the genes for CXCL1 and CXCL8 by activating the NF-κB signaling pathway, IL-17 appears to act mainly by stabilizing their mRNAs (19, 20). Intriguingly, however, IL-17 negatively regulates TNF-α-elicited production of other chemokines including CCL5 (RANTES) (23, 24), CX3CL1 (fractalkine) (25), and CCL27 (cutaneous T cell-attracting chemokine or CTACK) (26). Moreover, in a mouse model of allergic asthma, IL-17 functions as a negative regulator by repressing expression of the eosinophil chemokine CCL11 (eotaxin) and Th2 chemokine CCL17 (thymus- and activation-regulated chemokine or TARC) (27). Very little is known at present regarding the mechanism(s) for the repressive effects of IL-17 on these chemokines.

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The chemokines CXCL9, 10, and 11 act via the CXCR3 receptor, which is expressed by Th1 cells, and these three chemokines play a role in recruiting Th1 cells to a site of inflammation (28, 29). In inflammatory bowel disease, the epithelial cells lining the lumen of the gastrointestinal tract are an important source of chemokines (13, 30, 31). We show here that IL-17 represses TNF-α-mediated chemokine expression, which is expressed by Th1 cells, and these three chemokines may contribute to the recruitment of Th1 cells. In contrast, IL-17 synergizes with TNF-α to increase expression of CCL20, a chemokine that promote recruitment of Th17 cells.

Materials and Methods

Materials

Protein kinase inhibitors, SB203580, U0126, and tyrphostin AG1478 were obtained from Sigma-Aldrich. Stock solutions of the inhibitors were prepared in DMSO. Human recombinant TNF-α (210-TA) and IL-17A (317-TA) were obtained from R & D Systems. The RNA polymerase II inhibitor DRB (5,6-dichloro-1-b-D-ribofuranosylbenzimidazole) was from Sigma-Aldrich. The NF-κB-luciferase reporter had luciferase transcription under control of a promoter with three consensus NF-κB binding sites cloned upstream from a TATA box (32). The CMV-luciferase reporter had luciferase transcription under control of a fragment extending from −525 to +97 of the CXCL10 gene promoter (CXCL10/luciferase reporter; obtained from R. Ransohoff, Cleveland Clinic, Cleveland, OH) (33), a 1521-bp fragment of the CXCL10 gene promoter (CXCL10/luciferase reporter; obtained from R. Ransohoff, Cleveland Clinic, Cleveland, OH) (34), and a 400-bp region of the Ido1 promoter (obtained from K. Yamamoto, University of California, San Francisco, CA) (35, 36).

Cell culture

HT-29 CL19A human colorectal cancer cells were cultured in McCoy’s 5A medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (250 μg/ml) (13). CD4+ human T cells enriched for IL-17-producing cells were prepared as described previously (37) with minor modifications. Briefly, CD4+ T cells were isolated from PBMCs from healthy volunteers using a RosetteSep human CD4+ T cell isolation kit (StemCell Technologies) followed by a whole CD4+ T cell isolation kit (Miltenyi Biotec). Cells obtained in this manner consisted of 98% pure CD4+ T cells, of which approximately half were CD45RA+ and half were CD45RO+.

Chemotaxis assays

For collection of conditioned medium from HT-29 cells, densely confluent monolayer cultures of HT-29 cells in McCoy’s 5A medium plus 0.5% FBS were treated with nothing (control), TNF-α, IL-17, or TNF-α + IL-17 for 24 h. The medium was collected, centrifuged for 2 min at 13,200 rpm to remove cell debris, and stored at −80°C.

Chemotaxis assays were performed in Transwells (5-μm pore size, 6.5-mm diameter, 12-well plates; Corning). Culture human T cells (300,000 cells in 100 μl of medium) were placed in the top well and 600 μl of HT-29 conditioned medium was placed in the bottom well. The Transwells were incubated at 37°C for 3.5 h, after which the cells that migrated to the lower chamber were collected by centrifugation and stimulated for 5 h with PMA (30 ng/ml; Sigma-Aldrich), ionomycin (750 nM; Sigma-Aldrich), and GolgiStop (as described in the manufacturer’s protocol; BD Biosciences). Cells were then stained with allophycocyanin-Cy7-conjugated anti-human CD4 Ab (BD Biosciences; catalog no. 557871). Next, cells were fixed with 4% paraformaldehyde and permeabilized using a BD Cytofix/Cytoperm kit (BD Biosciences; catalog no. 554715). After permeabilization, cells were stained with PE-conjugated anti-human IL-17 (eBioscience; catalog no. 12-7179-73) and allophycocyanin-conjugated anti-human IFN-γ (BD Biosciences; catalog no. 554702) Ab. Samples were subjected to FACS analysis using a BD LSR flow cytometer and FlowJo software. The migration index was determined by setting the mean for the percentage of cells migrating into medium from untreated HT-29 cells at 1.0.
FIGURE 2. Assay of chemokine activity in medium conditioned by untreated HT-29 cells and cells treated with TNF-α and/or IL-17. Chemotaxis assays were performed with human CD4+ T cells using Transwells. The percentage of migrated cells that were IL-17 IFN-γ (A), IL-17 IFN-γ (B), and IL-17 IFN-γ (C) was determined by FACS analysis, and migration index as calculated as described in Materials and Methods. The actual mean percentage of cells migrating into medium from untreated HT-29 cells was 28.8% IL-17 IFN-γ, 1.3% IL-17 IFN-γ, and 1.3% IL-17 IFN-γ. Each bar represents the mean of results obtained with medium from two independent HT-29 cultures, and error bars represent the range for the two determinations.

Gene expression experiments

HT-29 cells were plated in 6-well plates at density of 3–5 million cells per well in McCoy’s 5A medium with 10% FBS plus antibiotics. Before treatment with cytokines, cells were incubated in medium with 0.5% FBS overnight. The next morning, cells were treated with TNF-α and/or IL-17. Following treatment with cytokines, cells were harvested, and RNA was prepared using the RNeasy Mini kit (Qiagen). RNA samples (2 μg) were reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (M02538; New England Biolabs). Real-time PCR was performed using SYBR Green (Applied Biosystems) for detection and the standard curve method for quantification, as described previously (40).

Promoter activity assay

For transfection experiments, HT-29 cells were plated at a density of 4 × 105 cells per 3.5-cm diameter well and transfected using Lipofectamine 2000 (Invitrogen). In all experiments, the plasmid pCMV-βgal, in which the β-galactosidase gene is under control of the CMV enhancer plus promoter, was cotransfected along with the luciferase reporter as an internal control for transfection efficiency and for the specificity of the effects observed (13). To determine the effect of TNF-α and IL-17 on promoter activity, cells were transferred to medium with 0.5% serum for 14 h before cytokine addition. IL-17 (final concentration of 50 ng/ml) was added to each culture 30 min before the addition of TNF-α (final concentration of 25 ng/ml). Incubation was continued for 6 h following TNF-α addition, after which cells were harvested and assayed for luciferase and β-galactosidase activity as described previously (13). The luciferase activity was then normalized to the β-galactosidase activity of the same extract. In some experiments cells were treated with PMA rather than TNF-α to activate reporter expression.

Phospho-ERK and tyrosine phosphorylation analyses

Following treatment with cytokines, cells were harvested in 1% Triton X-100 lysis buffer in TBS (15 mM Tris, 150 mM NaCl (pH7.6)) with protease and phosphatase inhibitors and cleared by centrifugation. For phospho-ERK analysis, samples were stored in Laemmli sample buffer at 80 °C. For epidermal growth factor receptor (EGFR) phosphorylation, samples were incubated with 4 μg/ml anti-EGFR (Upstate Biotech, catalog no. 06-847) for 2 h at 4 °C, after which immune complexes were isolated with 40 μl of protein A-agarose, washed, and eluted in Laemmli sample buffer. Protein samples were run on a 10% SDS-polyacrylamide gel and transferred to Immobilon-FL (Millipore) membranes. For detection of ERK, the membranes were probed with both anti-phospho-ERK (Cell Signaling Technology, catalog no. 9106S) and anti-total ERK-1 (Santa Cruz Biotechnology, catalog no. sc-93) Abs followed by IRDye 800CW and IRDye 680-conjugated secondary Abs (LI-COR Biosciences). For p38, the membranes were probed with both anti-phospho-p38 (Cell Signaling Technology, catalog no. 9211S) and anti-total p38 (Santa Cruz Biotechnology, catalog no. sc-535G) primary Abs followed by IRDye 800- (Rockland) and Alexa Fluor 680-conjugated (Molecular Probes) secondary Abs. For EGFR phosphorylation, membranes were probed with anti-PY99 (Santa Cruz Biotech, catalog no. sc-7020) and anti-EGFR. The membranes were then scanned using the Odyssey infrared imaging system (LI-COR Biosciences). Fold phosphorylation was determined from the integrated intensity of individual bands using the LI-COR Odyssey software; phospho-protein levels were normalized to total protein levels in each case.

ERK nuclear localization

HT-29 cells were cultured on glass coverslips. Cells were transfected with an expression construct for rat ERK2 fused with GFP on the C terminus in the pEGFPN1 vector (41) using Lipofectamine 2000 (Invitrogen). Transfected cells were preincubated in IL-17 30 min before the addition of TNF-α. Four hours after adding TNF-α, coverslips were removed, the cells
were fixed, and the coverslips were mounted onto slides using Vectashield mounting medium with 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). The cells were then imaged using an Eclipse TE2000U inverted microscope (Nikon).

Statistics
For comparisons of more than two means, data were subjected to one-way ANOVA followed by the Student-Newman-Keuls multiple comparison test. For comparison of two means, data were analyzed by the t test.

Results
IL-17 negatively regulates some chemokine genes by repressing transcription and positively regulates others by enhancing mRNA stability

The effect of IL-17, TNF-α, and IL-17 plus TNF-α on mRNA abundance for six chemokines is shown in Fig. 1. Two distinct patterns of regulation by IL-17 were observed. For CXCL10, CXCL11, and CCL5, IL-17 alone had no significant effect on gene expression, but IL-17 strongly repressed the induction of these three genes by TNF-α (Fig. 1, A–C). For CXCL8, CXCL1, and CCL20, IL-17 alone had no significant effect on gene expression, but it strongly synergized with TNF-α in inducing expression of these three genes (Fig. 1, D–F). All six chemokine genes have at least one binding site for NF-κB in their promoter elements (33–36, 42–44), and in each case NF-κB is thought to play an important role in the induction of the genes by proinflammatory cytokines, including TNF-α. The effect of IL-17, TNF-α, and IL-17 plus TNF-α on another well-studied NF-κB target gene, that encoding IκBα, was also examined. IL-17 alone had no significant

![Graph A](image1)

**FIGURE 4.** Effect of IL-17 on CXCL10 and CXCL8 mRNA stability. HT-29 cells were treated with IL-17 (50 ng/ml) (○) or not treated with IL-17 (●) for 30 min followed by addition of TNF-α (25 ng/ml) to all cultures. Cells were incubated for an additional 6 h. DRB (40 μM) was then added to each culture, and cells were harvested at 0, 15, 30, 60, 120, and 360 min after the addition of DRB. Abundance of CXCL8 (A) and CXCL10 (B) mRNA was determined by real-time PCR. Each point represents the mean ± SE of at least three replicate cultures. *, Mean differs significantly from mean of control cultures not treated with IL-17, p < 0.05.

![Graph B](image2)

**FIGURE 5.** Effect of IL-17 on ERK and p38 MAPK activity. HT-29 cells were treated for the indicated times with TNF-α (○), IL-17 (▲), or TNF-α plus IL-17 (●). Western blots were prepared and analyzed for phospho-ERK and total ERK (A) or phospho-p38 and total p38 (B). Results for phospho-ERK were normalized to total ERK (C), and those for phospho-p38 were normalized to total p38 (D). In C and D, each point represents the mean of two experiments; error bars show the range for the two experiments.
effect on expression of the IκBα gene, but it augmented TNF-α-induced expression (Fig. 1G).

We next used a functional assay to examine the effect of TNF-α and IL-17 on chemokine production by HT-29 cells. The HT-29 cells were treated with TNF-α and/or IL-17 for 24 h. The conditioned medium was harvested and used as a source of chemokines in chemotaxis assays of cultured human T cells. (Fig. 2). Consistent with the effect of TNF-α and IL-17 on CCL20 mRNA expression, TNF-α stimulated production of chemokine(s) that were chemoattractive to Th17 cells, and chemokine production was enhanced by the coaddition of IL-17 (Fig. 2A). Interestingly, the same response was observed with IL-17+ IFN-γ+ cells, which also express CCR6, the receptor for CCL20 (4) (Fig. 2B). In the same unconcentrated conditioned medium samples, there was not a sufficient amount of chemokines that attract Th1 cells for us to detect the effect of TNF-α or IL-17 on Th1-attractive chemokine production (Fig. 2C).

To determine whether the effects illustrated in Fig. 1 involved transcriptional or posttranscriptional regulation of gene expression, the effect of IL-17 and TNF-α was determined in cells transfected with luciferase reporter constructs in which luciferase transcription was directed by the CXCL10, CXCL8, or IκBα promoter. IL-17 alone had no significant effect on the CXCL10 reporter, but it strongly repressed TNF-α-induced expression (Fig. 3A), consistent with a transcriptional mechanism for the repressive effect of IL-17 on CXCL10 mRNA. IL-17 also modestly repressed TNF-α-induced expression of the CXCL8 and IκBα reporters (Fig. 3, B and C), suggesting that the augmentation of TNF-α-induced expression of CXCL8 and IκBα mRNA was posttranscriptional. The effect of IL-17 on the expression of a reporter in which luciferase expression is directed by three consensus NF-κB binding sites cloned upstream from a TATA box was also determined (Fig. 3D). In this functional assay, IL-17 alone had no significant effect on NF-κB activity, and it had a small negative effect on TNF-α or PMA-elicted activation of NF-κB.

The effect of IL-17 on CXCL10 and CXCL8 mRNA stability is shown in Fig. 4. CXCL8 mRNA was very unstable in HT-29 cells, and it was significantly stabilized in cells treated with IL-17 (Fig. 4A). In contrast, CXCL10 mRNA was very stable, and its stability was unaffected by IL-17 (Fig. 4B). Taken together with the results presented in Figs. 1 and 3, these results indicate that in the HT-29 cells IL-17 represses the expression of some chemokine genes by a transcriptional mechanism and increases the expression of others by increasing mRNA stability.

**FIGURE 7.** ERK and p38 MAPK inhibitors reverse the effects of IL-17 on CXCL10 and CXCL8 gene expression. SB203580 (10 μM), U0126 (1 μM), or DMSO vehicle was added to cultures 30 min before cytokine addition. IL-17 was added, followed immediately by TNF-α, and incubation was continued for 6 h. Cells were harvested, and abundance of CXCL10 (A) or CXCL8 (B) mRNA was quantified by real-time PCR. The mean of the untreated control cultures was set at 1.0. Each bar represents the mean ± SE of results obtained with three replicate cultures. Means with different letters were significantly different, p < 0.05.
Transcriptional repression and mRNA stabilization by IL-17 both depend on ERK and p38 MAPK activation

Although the signaling pathways for IL-17 action are not completely understood, evidence indicates that some effects are mediated by activation of ERK and/or p38 MAPK (19). The effect of TNF-α, IL-17, and TNF-α plus IL-17 on ERK (Fig. 5, A and B) and p38 MAPK (Fig. 5, C and D) was determined in HT-29 cells. In these cells, TNF-α transiently activated both ERK and p38 MAPK, whereas IL-17 alone had very little effect. The combination of IL-17 and TNF-α gave synergistic transient activation and also prolonged activation of both ERK and p38 (Fig. 5).

The effect of IL-17 on nuclear localization of ERK was examined by transfecting HT-29 cells with a GFP-ERK expression construct. The results indicated that the combination of IL-17 and TNF-α gave persistent (4 h) nuclear localization of ERK, whereas neither cytokine alone had this effect (Fig. 6). Taken together, the results presented in Figs. 5 and 6 indicate that IL-17 synergizes with TNF-α in activating ERK and p38 and also in promoting persistent nuclear localization of ERK.

We next used the selective protein kinase inhibitors SB203580, which inhibits p38, and U0126, which inhibits the ERK signaling pathway, to determine whether either or both kinases mediate the effects of IL-17 on CXCL10 and CXCL8 gene expression. The results (Fig. 7) indicated that SB203580 reversed both the repressive effect of IL-17 on CXCL10 and the inductive effect on CXCL8. U0126 reversed the repressive effect of IL-17 on CXCL10 and partially reversed the inductive effect on CXCL8. To determine whether the inhibitors reversed the repressive effect of IL-17 on CXCL10 promoter activity, experiments were performed with HT-29 cells transfected with the CXCL10/luciferase reporter construct. The results (Fig. 8) indicated that both SB203580 and U0126 partially reversed repression by IL-17 of the CXCL10 promoter. Finally, U0126 slightly increased the activity of the CXCL10 promoter above that seen with TNF-α alone (Fig. 8B), and the combination of TNF-α plus IL-17 plus U0126 gave stronger induction of CXCL10 mRNA than TNF-α alone (Fig. 7A). This result suggested that the relatively high baseline of ERK signaling in the HT-29 cells, attributable to the presence of a BRAF
mutation in these cells (45), might have a repressive effect on CXCL10 gene transcription that was relieved by U0126. In sum, the results presented in Figs. 7 and 8 demonstrated that the effects of IL-17 on both CXCL8 and CXCL10 gene expression were mediated, at least in part, by the ERK and p38 MAPK signaling pathways.

Biological effects of IL-17 are mediated in part by EGFR signaling

The results described above indicated that the effects of IL-17 on chemokine gene expression were partly mediated through the ERK signaling pathway. The activation of ERK by IL-17 in HT-29 cells is consistent with several previous studies performed with other cells (46, 47); however, the mechanism by which IL-17 activates ERK is not known. Recent studies have demonstrated that in human cells IL-17A acts via IL-17RA and IL-17RC receptors (48). IL-17RA has a very large cytoplasmic domain, and the IL-17 receptor family is not homologous to other known cytokine receptors. We considered the possibility that in colonic epithelial cells IL-17 binding to its receptor might trans-activate the EGFR, resulting in activation of the ERK signaling pathway. To test the hypothesis that EGFR mediated the effects of IL-17, we tested the ability of the specific EGFR kinase inhibitor tyrphostin AG1478 to reverse the effects of IL-17 on CXCL8 and CXCL10 gene expression. The results indicated that AG1478 partially reversed the effects of IL-17 on CXCL8 and CXCL10 mRNA abundance (Fig. 9, A and B) and CXCL10 promoter activity (Fig. 9C). Direct assays of EGFR phosphorylation after 5 min and 4 h of cytokine treatment showed that IL-17 and TNF-α synergized to promote phosphorylation of EGFR at 4 h (Fig. 10). The activation of EGFR by IL-17 plus TNF-α at 4 h was consistent with the prolonged activation of the downstream ERK signaling pathway. Thus, IL-17 in combination with TNF-α transactivates EGFR, and the inhibitor experiments indicate that the effects of IL-17 on CXCL8 and CXCL10 gene expression are in part mediated by EGFR transactivation.

Discussion

We show here that IL-17 strongly represses expression of the CXCR3 chemokines CXCL10 and 11 by HT-29 colonic epithelial cells. In addition, consistent with results reported previously with other cells (23, 24), IL-17 also represses CCL5 expression by HT-29 cells. CXCR3 is expressed on Th1 but not Th2 or Th17 cells; thus the CXCR3 ligands CXCL10 and 11 are selectively chemotactic for Th1 cells (28). CCL5 is also chemotactic for Th1 but not Th2 cells (49). Interestingly, negative regulation of CX3CL1 by IL-17 in ocular epithelial cells has also been reported (25). Like CXCL10, CXCL11, and CCL5, CX3CL1 is selectively chemotactic for Th1 cells and acts as an amplifier of polarized Th1 responses (50). It appears, therefore, that accumulation of IL-17-producing cells at the site of inflammation would attenuate subsequent recruitment of Th1 cells. There are two contexts in which this could occur. First, in the intestinal innate immune response IL-23 produced by dendritic cells and macrophages stimulates production of a family of proinflammatory cytokines, including TNF-α and IL-17, by γδ T cells and other innate immune cells (5, 15–18). Under these conditions it may be desirable to inhibit or delay recruitment of Th1 cells. Second, IL-17 produced by Th17 cells as they accumulate at the site of inflammation may negatively regulate the recruitment of Th1 cells. Consistent with the notion of a feedback loop in which IL-23 and IL-17 negatively regulate the IL-12/IFN-γ network, the administration of an anti-IL-17 mAb worsens the course of dextran sodium sulfate-induced colitis, the elimination of IL-23 worsens the development of T cell-mediated trinitrobenzene sulfonic acid-induced colitis, and donor Th17 cells...
negatively regulate Th1 differentiation and ameliorate acute graft-vers-host disease (51–53). Similarly, in a mouse model of allergic asthma, IL-17 functions as a negative regulator by repressing expression of the eosinophil chemokine CCL11 (eotaxin) and the Th2 chemokine CCL17 (thymus- and activation-regulated chemokine or TARC) (27). Taken together, these results are consistent with negative regulation by IL-17 of both Th1 and Th2 recruitment.

We also confirm and extend the previous observation that IL-17 cooperates with TNF-α to up-regulate expression of other chemokines, including CXCL8, CXCL1, and CCL20 (19–22). TNF-α plus IL-17 induced a remarkable 845-fold increase in CCL20 mRNA abundance (Fig. 1). CCL20 and its receptor CCR6 play a role in lymphorganogenesis in the intestine and are also thought to participate in the inflammatory response (54). Recent studies have demonstrated that CCR6 is expressed on human Th17 and Th1/Th17 cells (4, 7, 9). CCL20 stimulates calcium flux (4) and is chemotactic (9) for Th17 but not Th1 cells. Thus, our results suggest that accumulation of IL-17-producing cells at the site of inflammation would establish a positive feedback loop leading the recruitment of additional IL-17-producing Th17 cells. Interestingly, the CCL20 receptor CCR6 is expressed on CD4+CD25high Foxp3+ Treg cells, and CCL20 is chemotactic for these cells as well as for Th17 cells (55). The recruitment of Treg cells along with Th17 cells may serve to restrain the inflammatory response, thereby preventing severe tissue damage.

Consistent with an earlier report (19), CXCL8 is encoded by an unstable mRNA. The induction of CXCL8 gene expression by IL-17 was posttranscriptional and involved CXCL8 mRNA stabilization. TNF-α induction of the CXCL8 luciferase reporter was actually slightly decreased in IL-17-treated cells, although we cannot conclusively rule out a possible positive effect of IL-17 on CXCL8 transcription mediated by a cis-acting element outside the 1521-bp CXCL8 core promoter used for construction of the reporter. In contrast, our results indicate that CXCL10 is encoded by a very stable mRNA, the decay of which is not affected by IL-17. Rather, the negative effect of IL-17 on CXCL10 gene expression occurs via transcriptional repression, in agreement with the previously reported repression by IL-17 of CCL5 gene transcription (23).

Intriguingly, although IL-17 induces CXCL8 by stabilizing its mRNA and negatively regulates CXCL10 by repressing its transcription, both forms of regulation are dependent on the p38 and ERK MAPK signaling pathways. Like many other unstable mRNAs, CXCL8 mRNA contains an AU-rich element in its 3’-untranslated region. Stabilization of AU-rich element-containing mRNAs by p38 MAPK is thought to result from phosphorylation of the mRNA-destabilizing protein tristetraplin by the p38-activated kinase MK-2 and a consequent change in the subcellular location of tristetraplin mediated by binding to 14-3-3 (56). In contrast, relatively little is known regarding the mechanism for stabilization of mRNAs by the ERK signaling pathway.

As illustrated in Fig. 3, IL-17 modestly repressed TNF-α-mediated activation of the CXCL8 and IκBα promoters, two well known NF-κB targets (36), as well TNF-α-mediated activation of an NF-κB reporter. In contrast, IL-17 more strongly repressed the CXCL10 promoter. These results are consistent with a general weak interference with TNF-α activation of NF-κB targets plus additional selective repression of the CXCL10 promoter. The molecular mechanisms for these effects and the role of p38 and ERK signaling remain to be elucidated. Regarding the slight general interference with TNF-α activation of NF-κB, the synergistic induction of IκBα by IL-17 in combination with TNF-α is of interest. The NF-κB inhibitor IκBα is rapidly degraded in response to TNF-α, leading to nuclear entry and transcriptional activation by NF-κB. As an NF-κB target, IκBα is subsequently resynthesized, leading to termination of the NF-κB response. Because the coaction of IL-17 with TNF-α potentiates the induction of IκBα, it is possible that NF-κB-mediated transcription is halted earlier or more completely, thereby explaining the modest general inhibitory effect of IL-17 on NF-κB targets.

The molecular mechanism for selectively stronger repression by IL-17 of the CXCL10 promoter is also of considerable interest and remains to be explored further. It is possible that this selectivity is related to the specific coactivator complexes, including Bcl-3 and CARM1, required for the induction of CXCL10 gene expression by TNF-α (57, 58). The CXCL11 gene is located on the long arm of chromosome 4, in tandem with and ~10 kb telomeric to the CXCL10 gene (59). Although CXCL11 transcription has not been as thoroughly studied as CXCL10 transcription, it is of interest to note that the CXCL11 promoter has an NF-κB site with exactly the same sequence as the distal CXCL10 NF-κB site and a sequence resembling a likely IFN stimulatory response element (ISRE) located upstream of this site (Fig. 11). CCL5 gene transcription is also repressed by IL-17 (23), and the CCL5 promoter has an NF-κB site with two ISREs located upstream of this site. (Fig. 11). Thus, it is possible that strong transcriptional repression of CXCL10, CXCL11, and CCL5 by IL-17 is related to this shared promoter configuration.

The effects of IL-17 on chemokine gene expression were partially reversed by the specific EGFR tyrosine kinase inhibitor tyrphostin AG1478, suggesting that IL-17 signaling involves activation of the EGFR kinase. Consistent with this hypothesis, IL-17 cooperated with TNF-α in transactivating EGFR. The IL-17RA receptor has a very long cytoplasmic domain, and signaling pathways activated by IL-17 receptors are not completely understood. To our knowledge, this is the first observation implicating EGFR activation in IL-17 signaling, and this may account for the effect of IL-17 on ERK activity. Many G protein-coupled receptors activate

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**FIGURE 11.** Sequences of CXCL10, CXCL11, and CCL5 gene promoters. Sequences are from the human genome database (genome.ucsc.edu). NF-κB binding sites, ISREs, and TATA-like elements are bold and underlined. The NF-κB binding sites and ISREs of the CXCL10 and CCL5 promoter elements have been described previously (33, 42). Likely NF-κB and ISRE elements in the CXCL11 promoter were located by searching for sequences resembling the consensus elements.
EGFR and its downstream pathways including ERK, a phenomenon known as transactivation (60). Mechanisms for transactivation include extracellular release of EGFR ligands such as heparin-binding epidermal growth factor-like growth factor (HB-EGF) or TGF-α, which bind to and activate the EGFR, and activation of Src kinase, which phosphorylates Tyr^600 of EGFR, resulting in EGFR activation (60). TNF-α has been shown to transactivate EGFR in some epithelial cells (61) and to stimulate extracellular release of the EGFR ligand TGF-α and activate ERK in HT-29 cells (62). Our results confirm activation of ERK by TNF-α in the HT-29 cells. Although IL-17 alone had a very modest effect on ERK, it potentiated the early effect of TNF-α and caused more prolonged ERK signaling (Figs. 5 and 6).

In sum, our results indicate that IL-17 functions in concert with TNF-α to regulate chemokine expression in colonic epithelial cells. IL-17 inhibits expression of Th1-recruiting chemokines and simultaneously increases the expression of chemokines specific for Th17 and other immune cell types. Taken together with the earlier observations that the IFN-γ-positively regulates expression of chemokines that recruit Th1 cells (30, 63) and negatively regulates the Th17 chemokine chemokine CCL20 (9), these results suggest that expression of IL-17 at the site of inflammation would promote recruitment of Th17 and inhibit or delay recruitment of Th1 cells, whereas conversely the expression of IFN-γ would promote the recruitment of Th1 and inhibit the recruitment of Th17 cells.

Acknowledgments

We thank R. Ransohoff, A. Keates, and K. Yamamoto for providing the luciferase reporter constructs used in this project, J. Liu for assistance with the transfection experiments, and Robert Axtell and Shannon Dunn for helpful suggestions.

Disclosures

The authors have no financial conflict of interest.

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