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Selective Suppression of IL-12 Production by Chemoattractants

Michael C. Braun, Edward Lahey, and Brian L. Kelsall[1]

We investigated the ability of chemoattractants to affect IL-12 production by human monocytes and dendritic cells. We found that pretreatment of monocytes with macrophage chemoattractant proteins (MCP-1 to -4), or C5a, but not stromal-derived factor-1, macrophage inflammatory protein-1α, RANTES, or eotaxin, inhibited IL-12 p70 production in response to stimulation with *Staphylococcus aureus*, Cowan strain 1 (SAC), and IFN-γ. The production of TNF-α and IL-10, however, was minimally affected by any of the chemoattractants. The degree of inhibition of IL-12 p70 production by MCP-1 to -4 was donor dependent and was affected by the autocrine inhibitory effects of IL-10. In contrast, C5a profoundly suppressed IL-12 production in an IL-10-independent fashion. Neither TGF-β1 nor PGE_2 was important for the suppression of IL-12 by any of the chemoattractants tested. The accumulation of mRNA for both IL-12 p35 and p40 genes was inhibited by chemokine pretreatment. Interestingly, MCP-1 to -4 and C5a did not suppress IL-12 production by monocyte-derived dendritic cells (DC) stimulated with CD40 ligand and IFN-γ or by SAC and IFN-γ, suggesting that these factors may act at the site of inflammation to suppress IL-12 and IFN-γ production rather than in the lymph node to affect T cell priming. Despite the inability of C5a to inhibit IL-12 production by DCs, the receptor for C5a (CD88) was expressed by these cells, and recombinant C5a induced a Ca^{2+} flux. Taken together, these results define a range of chemoattractant molecules with the ability to suppress IL-12 production by human monocytes and have broad implications for the regulation of immune responses in vivo. *The Journal of Immunology*, **2000, 164: 3009–3017.**

Interleukin-12 is a heterodimeric cytokine composed of two disulfide-linked subunits, p35 and p40, which induces IFN-γ production by NK cells and T cells and is vital for the induction of Th1 T cell responses (reviewed in Refs. 1 and 2). The genes for the p35 and p40 chains of IL-12 are located on separate chromosomes (3) and are regulated independently. The IL-12 p35, but not the p40, chain is constitutively produced at a low level by a variety of cell types, and transcription of both genes as well as production of IL-12 p70 heterodimer are inducible (4–6). IL-12 is normally produced by monocyte/macrophages and dendritic cells in response to infection by bacteria or parasites, or following exposure to their products. In this regard, LPS and fixed *Staphylococcus aureus*, Cowan strain 1 (SAC),[2] have been used extensively for the study of IL-12 production, as they have been shown to provide an important stimulatory signal for the transcription of both the p35 and p40 genes and the production of the active IL-12 p70 heterodimer. In addition to microbial products, engagement of CD40 by CD40 ligand (CD40L) expressed on activated T cells has been shown to be important in the induction of IL-12 production during cognate APC-T cell interactions (7–11). Finally, it has recently been shown that low m.w. fragments of hyaluronan that are produced in areas of acute inflammation can stimulate IL-12 production, thus providing a mechanism by which macrophages may be induced to produce IL-12 in the absence of either bacteria or T cells (12). IFN-γ provides an important costimulus for the production of IL-12 (13, 14). Thus, while IFN-γ alone has little direct effect on IL-12 production, in combination with LPS, SAC, or CD40L it can dramatically enhance p35 and p40 gene transcription as well as IL-12 p70 production. In studies of monocyte/macrophages stimulated in vitro with LPS, this augmentation is enhanced by preincubation with IFN-γ up to 24 h before the LPS stimulus (6).

In contrast to these positive regulators of IL-12 production, it has become clear that signals that inhibit IL-12 production from APCs may be important for the regulation of cell-mediated immune responses. Initially described were cytokine inhibitors of IL-12, such as IL-10, TGF-β1, IL-4, and IL-13, the latter two having more complex effects than the former, in that preincubation with these cytokines can also enhance IL-12 production (15, 16). Recently, the engagement of noncytokine receptors by microbes (17–19), IgG in the form of Ig-opsonized bacteria (20) or immune complexes (21), complement components (19, 20, 22), adrenergic agonists (23), and 1,25-dihydroxyvitamin D_3 (24) has been shown to inhibit IL-12 p70 production by human monocytes; the latter two factors were also shown to inhibit CD40-induced IL-12 production by human monocyte-derived dendritic cells. Interestingly, all these inhibitors, despite acting through discrete surface receptors, appear to inhibit IL-12 in a more or less selective fashion, in that the production of other proinflammatory cytokines, such as TNF-α, remain largely unaffected.

We previously demonstrated that Abs to complement receptor 3 (CR3, MAC-1, CD11b/CD18) as well as CR3 ligands, such as *Histoplama capsulatum* and iC3b-coated RBCs, suppress the ability of human monocytes to produce IL-12 in response to a variety of stimuli (22). Because the binding of ligands to CR3 depends on its surface conformation, we initially sought to determine whether chemoattractants known to stimulate CR3-mediated adhesion would affect the ability of CR3 ligands to inhibit IL-12 production by human monocytes. This possibility was suggested by a recent

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[2] Abbreviations used in this paper: SAC, *Staphylococcus aureus* Cowan strain 1; CD40L, CD40 ligand; MCP, monocyte chemoattractant protein; MIP, monocyte inflammatory protein; SDF, stromal-derived factor; DC, dendritic cell.

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report describing the ability of MCP-1 to enhance oral tolerance, possibly via its ability to inhibit IL-12 production (25). Somewhat surprisingly, by screening a panel of chemokines and natural chemoattractants for their ability to suppress IL-12 production, we found that a selective set of C-C chemokines, the macrophage chemoattractant proteins (MCP-1 to -4), as well as the natural chemoattractant C5a themselves, *i.e.*, in the absence of exogenous CR3 ligands, were potent inhibitors of IL-12 production by human monocytes. This study thus defines a new range of chemokines with suppressive effects on IL-12 production. In addition, it identifies the natural chemoattractant C5a as a particularly potent molecule that suppresses IL-12 production at the level of gene transcription. The findings with C5a contribute to the growing body of information suggesting that an inverse relationship exists between complement activation and the generation of Th1 T cell responses.

Materials and Methods

Reagents

The following recombinant human chemokines were obtained from PreproTech (Rocky Hill, NJ): MCP-1, MCP-2, MCP-3, MCP-4, RANTES, eotaxin, monocyte inflammatory protein-1α (MIP-1α), MIP-1β, and stromal-derived factor-1 (SDF-1) (lot-specific endotoxin concentrations were 0.03, 0.02, 0.03, 0.01, 0.001, 0.01, 0.06, and 0.001 EU/μg, respectively). Recombinant human C5a (lot-specific endotoxin concentration, 0.005 EU/μg) and indomethacin were obtained from Sigma (St. Louis, MO). SAC was supplied by Calbiochem (San Diego, CA). Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA). Recombinant human IL-4 and GM-CSF were purchased from Genzyme (Cambridge, MA). Neutralizing Ab to human TGF-β1 (polyclonal chicken Ig) and control Ab (normal chicken Ig), and neutralizing Ab to human IL-10 (clone 23738.11) and isotype control (clone 20116.11) were obtained from R&D Systems (Minneapolis, MN). Recombinant trimerized human CD40L (CD154) was provided by Immunex (Seattle, WA).

Isolation and stimulation of human monocytes

Human monocytes were obtained from normal healthy donors (total $n = 30$) by standard leukopheresis and purified by counterflow centrifugation (elutriation), which yielded cells of uniform forward/side scatter that were 95–99% $CD14^+$ by flow cytometry. Cells were cultured at a density of 2×10^6 cells/ml in 1 ml of RPMI 1640 (Biofluids, Rockville, MD) supplemented with 10% FCS (Biofluids), 100 μg/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, 5% NCTC-109 medium (Biofluids), 15 mM HEPES, and 200 mM glutamine (cRPMI) at 37°C and 6% CO_2. For measurement of cytokine production, human monocytes were preincubated with medium alone or varying concentrations of chemokines or C5a for 1 h at 37°C before stimulation with SAC (0.01%, w/v) and IFN-γ (100 ng/ml); 24 h later culture supernatants were collected and stored at −20°C until assayed for cytokines.

To determine the role of autocrine inhibitors of IL-12 in the monocyte cultures, elutriated monocytes were preincubated for 1 h with chemokines (100 nM), C5a (100 nM), or medium alone and one of the following: neutralizing Ab to TGF-β1 (10 μg/ml) or control Ab (10 μg/ml), neutralizing Ab to IL-10 (2.5 μg/ml) or isotype-matched control Ab (2.5 μg/ml), or indomethacin (1×10^{-5} M). SAC (0.01%) and IFN-γ (100 ng/ml) were then added; following 24 h of culture, supernatants were harvested and stored at −20°C until assayed for IL-12 p70 production. For studies with pertussis toxin, elutriated monocytes were cultured for 2 h with 250 ng/ml of purified pertussis toxin before incubation with chemokines and stimulation with SAC/IFN-γ as described above.

Generation and stimulation of human dendritic cells (DC)

DC were derived from elutriated monocytes as previously described (26). Briefly, monocytes were cultured for 7 days in cRPMI supplemented every other day with IL-4 (100 ng/ml) and GM-CSF (100 ng/ml). Nonadherent cells were harvested by gentle washing, and the majority (70–90%) were demonstrated by flow cytometry to express high levels of CD1a (clone HI149, PharMingen, San Diego, CA) and low levels of CD83 (clone HB15e, PharMingen), consistent with prior reports (26). Greater than 95% of the cells excluded trypan blue and demonstrated characteristic dendrite formation on examination with phase-contrast light microscopy. The DC were resuspended at a density of 1×10^6 cells/ml in cRPMI and treated with various chemokines (100 nM), C5a (100 nM), or medium alone for 1 h before stimulation with either SAC (0.01%) and IFN-γ (100 ng/ml), or CD40L (3 μg/ml) and IFN-γ (100 ng/ml). Supernatants were collected after 24 h of culture and stored at −20°C until assayed for cytokines.

Flow cytometry for C5a and IL-10 receptor expression

C5a receptor expression on monocyte-derived DC (after 7 days in culture with GM-CSF and IL-4) was performed as follows. Cells (1×10^6) in 100 μl of staining buffer (PBS containing 0.2% BSA and 0.1% sodium azide) were sequentially incubated with human IgG as a blocking agent for 15 min, followed by either FITC- or PE-conjugated isotype control Abs (PharMingen) or mouse anti-human CD88 FITC-conjugated mAb (clone W17/1, RDI, Flanders, NJ) or mouse anti-human CD1a PE-conjugated mAb (PharMingen) for 45 min. IL-10R expression was performed on 1×10^6 fresh elutriated human monocytes cultured for 1, 2, 4, 6, or 12 h with 100 nM MCP-1- to 4 or C5a with and without SAC/IFN-γ stimulation. At the given time, the cells were harvested, washed twice with ice-cold staining buffer, and stained with either PE-conjugated isotype matched control Ab (clone R35-95, PharMingen) or PE-conjugated rat anti-human IL-10R mAb (clone 3F9, PharMingen) as described above. All incubations were performed at 4°C in staining buffer, and cells were washed twice with staining buffer before and after incubations. The stained cells were analyzed on a FACScan flow cytometer using CellQuest software (Becton Dickinson, San Jose, CA).

Cytokine ELISAs

Cell culture supernatants were assayed for cytokines by ELISA using matched Ab pairs according to the manufacturer's suggestions. ELISA reagents for human IL-12 p70 were purchased from R&D Systems and for IL-10 and TNF-α from BioSource International (Camarillo, CA); the respective capture and detection Abs were as follows: IL-12 p70, clone 24945.11; and polyclonal goat IgG, IL-10 clones AHC8102 and AHC7109, and TNF-α clones AHC3712 and AHC3419. Briefly, the capture Ab was bound to 96-well ELISA plates (Immulon 4, Dynatech, Chantilly, VA) in the appropriate buffer overnight at 4°C; capture Ab for IL-12 p70 was used at a concentration of 4 μg/ml diluted in PBS; IL-10 and TNF-α capture Abs were used in a concentration of 2 μg/ml diluted in bicarbonate buffer. The plates were then washed (three times) with PBS with 0.05% Tween-20, and blocked for 2 h at room temperature; 1% BSA, 5% sucrose, and 0.05% sodium azide in PBS (pH 7.3) was used to block the IL-12 p70 plates, while 3% BSA in PBS was used for the remaining cytokines. Cytokine standards and supernatants were diluted as necessary; 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, and 0.1% BSA was used as the diluent for the IL-12 p70 assay, and the IL-10 and TNF-α, ELISAs used 3% BSA in PBS as a diluent. The ELISA plates were then incubated overnight at 4°C. The plates were washed, and bound cytokine was revealed with a biotin-labeled detecting Ab (2 h at room temperature). IL-12 p70 detection Ab was used at 300 ng/ml, while IL-10 and TNF-α detection Abs were used at 500 ng/ml. This was followed by HRP-conjugated streptavidin (Zymed, South San Francisco, CA; 1/1000 for 30 min at room temperature) and the substrate *o*-phenylenediamine dihydrochloride (Sigma) at 0.5 mg/ml in phosphate-citrate buffer (pH 5.0) and 0.03% H_2O_2. The OD of the individual wells was determined at 450 nm using an automated ELISA reader (Dynatech). The lower limit of sensitivity of the assays was 32 pg/ml for IL-12 p70 and IL-10, and 64 pg/ml for TNF-α. Assays for TGF-β1 were performed using ELISA kits from Genzyme according to the manufacturer's instructions. TGF-β1 levels were measured following acidification, and therefore reflect both the active and latent forms of TGF-β1.

RT-PCR

Elutriated monocytes (2.0×10^7) were stimulated for 16 h as described above in the presence or the absence of 100 nM MCP-1, MCP-2, MCP-3, MCP-4, or C5a. Total RNA was obtained using STAT-60 (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. RNA concentrations were determined by measuring the OD at 260 nm. mRNA for each experimental condition was reverse transcribed with oligo(dT) priming to first-strand cDNA using Superscript II reverse transcriptase (Life Technologies, Gaithersburg, MD). Briefly, 1 μg of total RNA suspended in RNase-free water was added to 16 μl of an RT reaction mixture consisting of 20 mM Tris-HCl, 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM DTT, and 1 mM dNTPs. After an initial incubation of 5 min at 42°C, 200 U of Superscript II RT was added, the reaction was continued for 50 min at 42°C and was then terminated at 70°C for 10 min. The samples were chilled on ice for 15 min. RNase H (2 U) was added, and the reaction was incubated for 20 min at 37°C. The first-strand cDNA was then stored at −80°C before PCR amplification.

Table I. *IL-12 p70 production by elutriated human monocytes stimulated with SAC (0.01%) and IFN-γ (100 ng/ml) with or without chemoattractants (100 nM)*[a]

Condition	IL-12 p70 (pg/ml)
SAC + IFN-γ	929.7 ± 421.8
+ SDF-1	867.7 ± 646.1
+ Eotaxin	872.9 ± 594.4
+ MIP-1α	589.9 ± 162.4
+ RANTES	831.0 ± 288.9
+ MCP-1	210.6 ± 158.1*
+ MCP-2	430.5 ± 468.0
+ MCP-3	159.9 ± 83.7*
+ MCP-4	256.1 ± 83.1*
+ C5a	55.5 ± 33.1*

[a] Values are means ± SD from cells of eight separate donors.
*, $p < 0.01$.

PCR amplification was performed using 2 μl of cDNA template and 50 μl of a reaction mixture consisting of 20 mM Tris-HCl, 50 mM KCl, 0.2 mM dNTPs, 1.25 mM $MgCl_2$, and 2.5 U Taq DNA polymerase (Life Technologies). The following primer pairs were used at a concentration of 1 μM: IL-12 p40: sense, 5′-AGAGGCTCTTCTGACCCCCAG-3′; antisense, 5′-CTCTTGCTCTTGCCCTGGACCTG-3′; IL-12 p35: sense, 5′-TCAGCA ACATGCTCCAGAAGGC-3′; antisense, 5′-TGCATTCATGGTCTTGAA CTCCACC-3′; and GAPDH: sense, 5′-TGAAGGTCGGAGTCAACGGATT TGGT-3′; antisense, 5′-CATGTGGGCCATGAGGTCCACCAC-3′. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 2 min, followed by cycles of 94°C for 45 s, 60°C for 60 s, and 70°C for 90 s and a final elongation step of 70°C for 5 min. The number of amplification cycles was determined by prior experiments to ensure linear phase amplification of cDNA template (data not shown). GAPDH was amplified for 25 cycles, IL-12 p40 for 30 cycles, and IL-12 p35 for 32 cycles. Amplified PCR products were resolved by gel electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

Statistics

Statistical analysis was performed by paired t testing using SigmaStat software (Jandel, San Raphael, CA).

Results

The MCPs and C5a selectively suppress IL-12 p70 production from human monocytes

We initially determined whether the exposure of elutriated human monocytes to chemoattractants in vitro could inhibit the production of IL-12 p70 production in response to a known IL-12 stimulus. Thus, we preincubated monocytes for 60 min with a panel of C-C (MIP-1α, RANTES, MCP-1 to -4, and eotaxin) and C-X-C (SDF-1) chemokines and natural chemoattractants (C5a). Following stimulation with SAC (0.01%, w/v) and IFN-γ (100 ng/ml) for 24 h, IL-12 p70 as well as other cytokines were measured in the culture supernatants by ELISA. As shown Table I, we found that MCP-1, MCP-3, MCP-4, and C5a significantly suppressed the production of IL-12 p70. MCP-2 also suppressed IL-12 p70 production, although this effect was more variable. Thus, despite significant suppression of IL-12 p70 production from cells from individual donors (Fig. 1), when multiple donors were included in the analysis (Table I) this suppression failed to reach statistical significance. Interestingly, the degree of IL-12 suppression by a given MCP and by the MCPs as a group was donor dependent, which may reflect interdonor variations in receptor-ligand affinity or postreceptor signaling pathways (Fig. 1). The MCP proteins were less suppressive overall compared with the consistent and virtually complete suppression of IL-12 by C5a (Fig. 1). The other chemokines tested had minimal to no effect on IL-12 p70 production. In addition, as might be expected, the higher the baseline IL-12 production (e.g., donor 2, Fig. 1), the less effect overall of

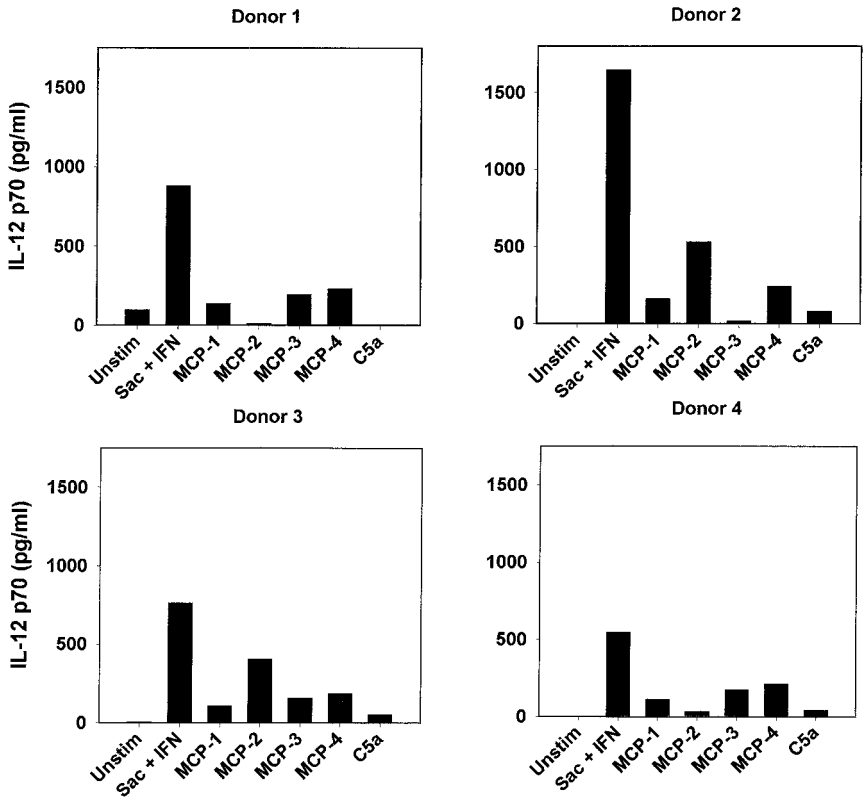

FIGURE 1. IL-12 p70 production from elutriated human monocytes stimulated with SAC (0.01%) and IFN-γ (100 ng/ml) in the presence or the absence of MCP-1 to -4 or C5a (100 nM). Values are means of duplicate assays for IL-12 p70 production from monocytes from four individual donors.

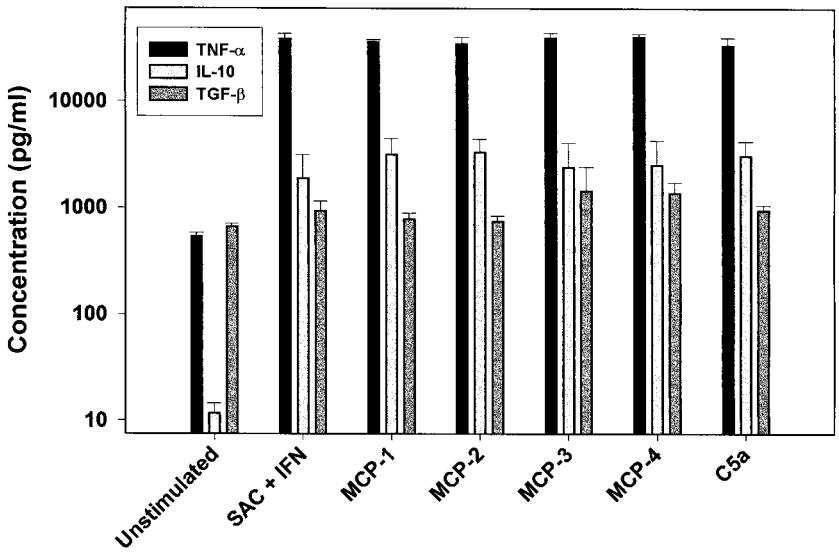

FIGURE 2. Production of TNF-α, IL-10, and TGF-β1 by monocytes following SAC (0.01%) and IFN-γ (100 ng/ml) stimulation with or without MCP-1 to -4 or C5a (100 nM) preincubation. Data are the mean ± SD from four separate donors ($p >$ 0.5 for all conditions).

the MCP proteins; however, even in this case C5a suppressed production by >80%.

We next determined whether IL-12 was suppressed in a selective fashion. As shown in Fig. 2, the production of IL-10, TGF-β1, and TNF-α was not significantly affected by any of the inhibitors. In addition, the chemokines and chemoattractants themselves did not directly stimulate the production of IL-10 or any other of the cytokines measured (data not shown). The fact that IL-10, TGF-β1, and TNF-α production was not affected suggested that the suppression of IL-12 seen was not due to any generalized toxic effects of the chemoattractants. Indeed, we found no significant differences between any of the culture conditions with respect to the total number of monocytes/macrophages present after 24 h in culture or the viability of the cultured cells, as determined both by trypan blue exclusion of cells directly in the culture dish and by flow cytometric analysis of propidium iodide-stained cells removed from the culture plates.

We next determined the dose-response curve for the suppression of IL-12 by C5a and MCP-1, the inhibitors with the most potent ability to suppress IL-12 p70 production. As shown in Fig. 3, the maximal suppressive effects of C5a occurred at a dose of less than 10 nM, and those of MCP-1 at concentrations <20 nM. These levels, as well as those of the other MCP proteins used, are consistent with prior reports of the doses required to induce chemotaxis and other biological functions (27, 28).

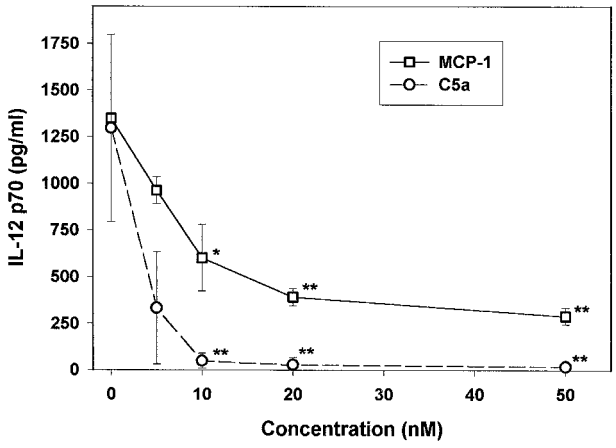

FIGURE 3. Production of IL-12 p70 by human monocytes following SAC (0.01%) and IFN-γ (100 ng/ml) stimulation in the presence of varying concentrations of either MCP-1 or C5a. Data are the mean ± SD from three separate donors (*, $p < 0.05$; **, $p < 0.01$).

C5a and MCP-1 suppress accumulation of mRNA for IL-12 p35 and p40

To determine whether these mediators suppress IL-12 production at the level of gene transcription, we performed semiquantitative RT-PCR for IL-12 p35 and p40 mRNA isolated from monocytes stimulated with SAC and IFN-γ with and without prior treatment with MCP-1 to -4 or C5a. As shown in Fig. 4, pretreatment with MCP-1 or C5a resulted in marked suppression of mRNA accumulation for both the p35 and p40 genes. MCP-2 to -4 had a more modest effect on mRNA accumulation, as is reflected by the protein data. This suggests that signaling by these chemokines and C5a acts at the level of gene transcription. Interestingly, C5a suppressed p35 transcription more than p40 transcription, similar to the effects of other IL-12 inhibitors, such as 1,25-dihydroxyvitamin D_3 (24).

Inhibition of IL-12 production by MCP-1 to -4 is partially dependent on IL-10

As the production of IL-10 and TGF-β1 in response to SAC and IFN-γ stimulation was largely unaffected by pretreatment with chemoattractants, we sought to determine whether chemoattractant pretreatment might be affecting monocyte sensitivity to autocrine

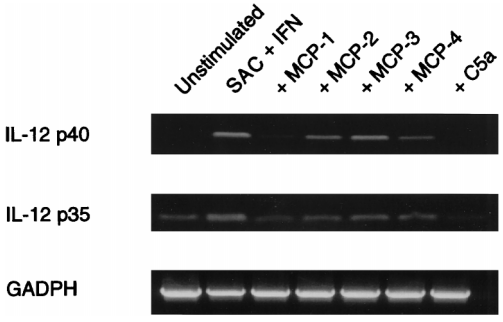

FIGURE 4. RT-PCR for accumulation of IL-12 p35, p40, and GADPH mRNA. Human monocytes were cultured for 16 h with medium alone, SAC (0.01%) and IFN-γ (100 ng/ml), or SAC (0.01%) and IFN-γ (100 ng/ml) and chemoattractants (100 nM).

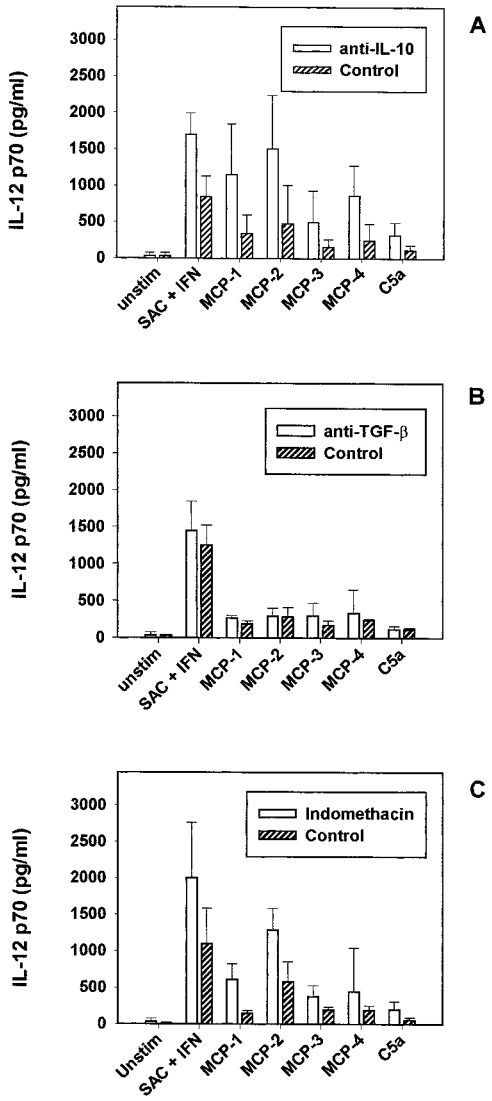

FIGURE 5. IL-12 p70 production from monocytes stimulated with SAC (0.01%) and IFN-γ (100 ng/ml) with or without preexposure to MCP-1 to -4 or C5a (100 nM). Monocytes were cultured under the following conditions: neutralizing Ab to IL-10 or isotype-matched control Ab (*A*), neutralizing Ab to TGF-β1 or control Ab (*B*), and with or without indomethacin (*C*). Data are the mean ± SD from three separate donors ($p > 0.05$ for all variables tested).

inhibition of IL-12 by those factors. Accordingly, we added neutralizing Abs to IL-10 or TGF-β1, or indomethacin, to prevent PGE_2 induction, before exposure to chemoattractants and stimulation with SAC and IFN-γ. As shown in Fig. 5*A*, anti-IL-10 partially reversed the suppression of IL-12 seen with MCP-1 to -4. Anti-IL-10 had minimal, but reproducible, effects on reversing the suppression seen with C5a. The reversal of suppression by anti-IL-10 with the MCP proteins was not statistically significant when multiple donors were combined for analysis (Fig. 5*A*) as there was significant interdonor variation in this effect. However, for cells from many individual donors this effect was remarkable. In contrast to anti-IL-10, neither anti-TGF-β1 nor indomethacin significantly reversed the inhibition induced by any of the chemoattractant proteins. We then pretreated monocytes with a combination of anti-IL-10, anti-TGF-β1, and indomethacin to determine whether there might be additive inhibitory effects of IL-10, TGF-β1, and PGE_2 induced by the chemoattractants. We found little enhancement of the reversal of inhibition over that seen with anti-IL-10

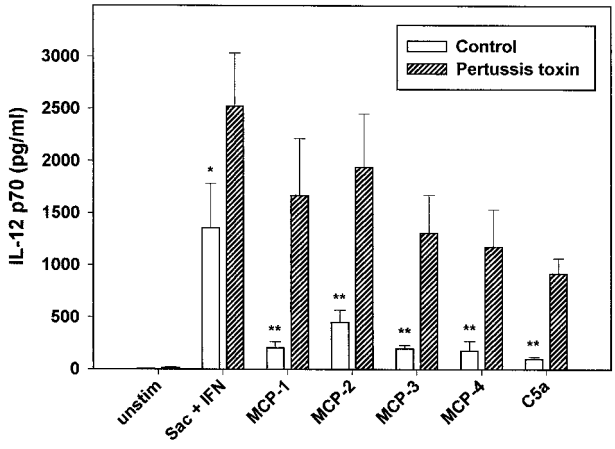

FIGURE 6. Effect of pertussis toxin on chemoattractant-mediated suppression of IL-12 p70. Monocytes were cultured for 2 h with 250 ng/ml of pertussis toxin and then stimulated with SAC (0.01%) and IFN-γ (100 ng/ml) in the presence or the absence of MCP-1 to -4 or C5a (100 nM). Data are the mean ± SD from three separate donors (*, $p < 0.05$; **, $p < 0.01$).

alone, demonstrating that IL-10 was predominantly responsible for this effect (data not shown). In addition, we performed initial studies to address the possibility that the sensitivity of donor cells to IL-10 might be affected by pretreatment with chemoattractants, as the levels of IL-10 produced in response to stimulation were similar with C5a and MCP-1 to -4 pretreatment, yet the degree of reversal with anti-IL-10 was remarkable only with select MCP proteins. We measured expression of the IL-10R on chemoattractant or medium-treated monocytes by flow cytometry. Pretreatment with MCP-1 to -4 or C5a did not affect cell surface receptor expression by monocytes directly or following stimulation with SAC and IFN-γ (data not shown), suggesting that differences in sensitivity to IL-10 may be due to alterations in postreceptor signaling.

Inhibition of IL-12 production by chemoattractants is sensitive to pertussis toxin

The chemoattractant proteins are known to transmit signals via seven-transmembrane domain receptors that couple to G proteins of the G_i and possibly G_q classes (29). To determine the extent to which the inhibitory signals for IL-12 production are dependent on signaling via G_i, we pretreated monocytes with the G_i inhibitor pertussis toxin before exposure to the chemoattractants and simulation with SAC and IFN-γ. As shown in Fig. 6, we found that pertussis toxin partially prevented the inhibition of IL-12 production chemoattractants. Despite using high doses of pertussis toxin (up to 500 ng/ml) and long preincubation times (up to 4 h), complete reversal of the inhibitory effects of any of the chemoattractants was never observed (data not shown).

MCP-1 to -4 and C5a do not inhibit IL-12 p70 production from DC

We next sought to determine whether the inhibition of IL-12 p70 production with MCP-1 to -4 and C5a seen with monocytes also applied to DC. For these experiments we cultured elutriated monocytes in GM-CSF and IL-4 for 7 days to obtain DC (26). In addition, we cultured these cells for an additional 3 days with either a monocyte-derived supernatant (26) or soluble CD40L trimer (30), which resulted in DC with a more mature or differentiated phenotype. We exposed both these populations of DC to a range of chemokines, including MCP-1 to -4 and C5a before stimulation

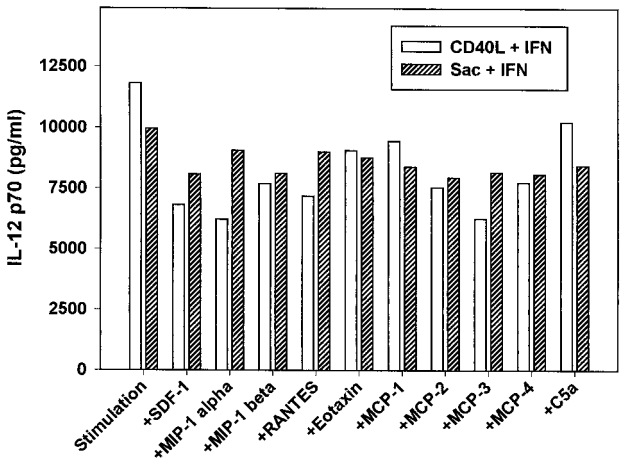

FIGURE 7. Effects of chemoattractants on IL-12 p70 production from monocyte-derived DC. Immature DC ($CD1a^{high}/CD83^{low}$) were stimulated with either SAC (0.01%) and IFN-γ (100 ng/ml) or CD40L (3 μg/ml) and IFN-γ (100 ng/ml) in the presence or the absence or various chemoattractants (100 nM). The figure is representative data from one of two experiments using DC derived from different donors.

with either CD40L and IFN-γ, or SAC and IFN-γ. As shown in Fig. 7, we found that none of the chemoattractants significantly suppressed the production of IL-12 p70 by the less mature cells in response to either stimulus. As shown in Fig. 8, this lack of suppression did not appear to be due to a lack of functional expression of chemokine receptors by these cells. C5a receptor (CD88) expression was clearly demonstrable by flow cytometry. In addition, MCP-1 to -4 as well as C5a readily induced an intracellular Ca^{+2} flux in the less mature DCs (data not shown), consistent with prior reports (31–33). Also consistent with prior studies, we found that maturation of DCs by exposure to either CD40L or monocyte-conditioned medium severely reduced their capacity to produce IL-12 p70 (34). Similar to the findings with the less mature DCs, however, incubation of the differentiated DCs with MCP-1 to -4 or C5a before stimulation had no effect on the minimal production of IL-12 p70 by these cells (data not shown).

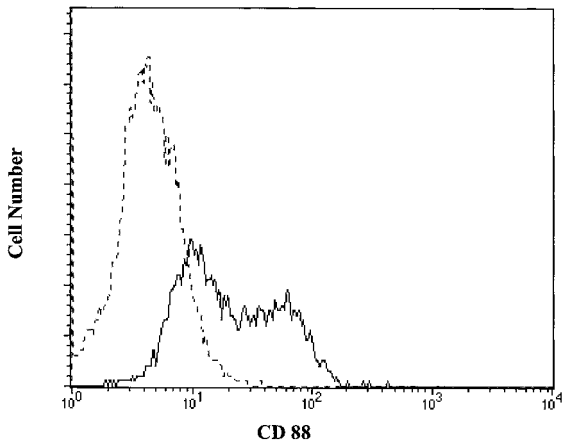

FIGURE 8. Expression of CD88 (C5a receptor) by $CD1a^+$ DC. Monocyte-derived DC were stained with FITC-conjugated anti-CD88 and PE-conjugated anti-CD1a or with isotype-matched control Abs and analyzed by flow cytometry. Shown is a histogram of staining with anti-CD88 (solid line) or isotype control Abs (broken line) following gating only on $CD1a^+$ cells. The figure is representative of one of three experiments using DC derived from different donors.

Discussion

IL-12 is a key regulatory cytokine for the generation of Th1 responses both in vitro and in vivo (1, 2). Thus, an understanding of its regulation is vital for understanding the development of T cell-dependent immune responses. We previously demonstrated that Abs to the β_2 integrin complement receptor 3 (CR3, MAC-1, CD11b/CD18) as well as CR3 ligands, such as *Histoplama capsulatum* and iC3b-coated RBCs, suppress the ability of human monocytes to produce IL-12, but not IL-6, TNF-α, TGF-β1, or IL-10, in response to a variety of stimuli (22). These findings suggest that the binding of iC3b-opsonized micro-organisms to CR3 on human monocytes or DC may provide a mechanism by which certain pathogens, such as *Leishmania* species (17, 18, 35), inhibit a host immune response that is important for their elimination. Because the binding of ligands to CR3 depends on its surface conformation, we initially sought to determine whether chemoattractants known to induce CR3-mediated adhesion would have effects on the inhibitory signals provided through CR3. Surprisingly, we found that a select group of chemoattractants themselves, i.e., in the absence of additional CR3 ligands, suppressed IL-12 production by human monocytes.

We initially screened a panel of chemokines from the C-C (MCP-1 to -4, MIP-1α, RANTES, and eotaxin) and C-X-C (SDF-1) chemokine families as well as the chemoattractant C5a for their abilities to affect the production of IL-12 as well as other cytokines by human monocytes in response to a well-known monocyte stimulus, SAC and IFN-γ. We found that many chemokines had insignificant effects on IL-12 production from monocytes isolated from multiple donors. This group included chemokines known to bind to the chemokine receptors CCR1 (MIP-1α), CCR3 (eotaxin, RANTES), CCR4 (MIP-1α, RANTES), and CCR5 (MIP-1α, RANTES) as well as to CXCR4 (SDF-1). Of these receptors, all are known to be expressed on human monocytes with the exception of CCR3, which cannot be detected in this cell type at the protein or mRNA level (36, 37).

In contrast, the MCP family of C-C chemokines (MCP-1 to -4), all of which bind to the CCR2-receptors CCR2a and CCR2b, inhibited IL-12 p70 production. This inhibition was specific, in that production of IL-10 and TNF-α was largely unaffected, and the degree of inhibition was donor dependent. In addition, as shown in Fig. 1, the degree of inhibition was MCP dependent, in that different members of the MCP family could have quite different effects on cells from a given donor. Finally, we demonstrated that the suppression of IL-12 by the MCP proteins is due in part to the effects of paracrine/autocrine production of IL-10 (and not TGF-β1 or PGE_2) by the stimulated mononuclear cells (Fig. 5). Because the levels of IL-10 in the culture supernatants were only minimally affected by the MCPs (Fig. 2), and the chemokines failed to either induce the production of IL-10 in the absence of other stimuli or directly alter IL-10R expression, it is possible that the reversal of suppression by anti-IL-10 reflects differences in individual donor sensitivity to IL-10.

The suppression of IL-12 by MCP family members is consistent with prior studies performed in mice. Thus, it has been shown that treatment of mice with MCP-1 results in the enhancement of systemic tolerance induced by oral protein feeding and that this correlated to a reduction in the production of IL-12 within mucosal tissues. In addition, it was shown that treatment with anti-MCP-1 prevented oral tolerance induction, which correlated with the enhanced production of IL-12 (25). This ability of MCP-1 to regulate IL-12 at mucosal surfaces is of particular interest for several reasons. Firstly, the presence of low levels of IL-12 appears to correlate with noninflammatory and tolerizing conditions within the

intestine, while the presence of high levels of IL-12 has the opposite effect (25, 38, 39). Secondly, intestinal epithelial cells produce a number of chemokines, including MCP-1 (40, 41), implying that these cells are a potential direct source of MCPs in the intestine. Finally, it has recently been shown that mice with targeted deletion of the G protein $G_i2\alpha$ develop a spontaneous Th1-mediated inflammatory bowel disease (42). In this model, inflamed colonic tissue expresses high levels of IL-12 p40 mRNA, and cultured intestinal tissue and purified intestinal T cells spontaneously produce high levels of IFN-γ and IL-1β. Since MCP-1 signals via a G-linked receptor and results in the suppression of IL-12 production, it is logical to hypothesize that $G_i2\alpha$ knockout mice develop Th1-mediated colitis, because they have poorly regulated production of IL-12. Taken together, these prior studies suggest that the production of MCP proteins within the intestine, possibly by epithelial cells, contributes to a generally suppressive environment at this site. That this suppressive environment is mediated by IL-10 is supported by the findings of the current study and is consistent with the results of prior studies demonstrating the presence of high levels of IL-10 within the intestinal tract (43–45). In addition, it is consistent with the fact that IL-10 mice develop Th1-mediated enterocolitis (46, 47).

Studies in mice also imply a role for MCP-1 in the regulation of IL-12 at nonmucosal sites. Thus, in a mouse model of endotoxic shock, it was shown that the administration of exogenous MCP-1 significantly protected mice from endotoxin-induced lethality (similar to the administration of anti-IL-12 in other studies (48, 49)), while the administration of anti-MCP-1 enhanced lethality (50). Importantly, enhanced lethality in this system correlated with high serum levels of IL-12 and low levels of IL-10, while its inhibition by MCP-1 correlated with high IL-10 and low IL-12 levels. Finally, it has been suggested that MCP-1 enhances the development of Th2 granuloma in the lungs of mice injected i.v. with eggs from *Shistosoma mansoni*, and that such enhancement may be partially related to its ability to suppress the production of IL-12 (51).

Our findings have significantly extended these prior murine studies. We now demonstrate the effects of MCP proteins on IL-12 production by human cells, show that the suppressive effect extends to four members of this family, MCP-1 to -4, and demonstrate that the degree of suppression is both donor and MCP dependent. In addition, we demonstrate that the mechanism of IL-12 suppression by such proteins is largely dependent on IL-10 and may be mediated not by absolute levels of IL-10, but by alterations in the sensitivity of cells to this cytokine.

In contrast to the variable effects of the MCPs, striking and consistent suppression of IL-12 p70 production was found with C5a, a classical chemoattractant that binds to the C5a receptor (29). Greater than 10-fold reduction in the production of IL-12 was consistently seen in all donors with C5a. The suppression of IL-12 by C5a was specific for IL-12 (Fig. 1), occurred maximally at concentrations as low as 10 nM (Fig. 3) and acted at the level of gene transcription for both p35 and p40 chains (Fig. 4), and as with the MCPs, autocrine production of TGF-β1 and PGE_2 appeared to have a minimal role (Fig. 5, *B* and *C*). In contrast to the MCPs, however, the suppression of IL-12 was only modestly reversed by anti-IL-10 (Fig. 5*A*). Thus, C5a appears to suppress IL-12 production directly or via the induction of a novel, as yet unidentified autocrine inhibitory factor(s). These findings with C5a are consistent with a recent paper by Wittman et al. (52), who showed that C5a suppressed IL-12 p40 and p70 production by IFN-γ-primed human monocytes stimulated with LPS. In contrast to this prior report, however, we found that suppression of IL-12 production following stimulation with SAC and IFN-γ is partially, although minimally, dependent on IL-10. In addition, we explored the ability of MCP-1 to -4 and C5a to inhibit the production of IL-12 p70 by DCs and determined that these chemoattractants had no effect on IL-12 p70 production by these cells. The lack of effect was not due to the inability of these agents to induce an intracellular calcium flux in DCs.

The inhibition of IL-12 by C5a initially appeared to contradict the facts that these agents are known to potently induce chemotaxis, phagocytosis, and respiratory burst capacities of human monocytes and neutrophils (29), and that blocking C5a activity with a neutralizing antiserum reduced mortality in a rat model of septic shock (53). These effects of C5a, however, are not necessarily inconsistent with the findings reported here. In fact, chemoattractants such as C5a as well as the MCP proteins may act to attract monocyte/macrophages into sites of acute inflammation and at the same time act to prevent potentially harmful effects of high levels of IL-12, such as acute toxicity, and the triggering of potentially deleterious autoreactive Th1 immune responses (1). In this fashion, these chemoattractants could act as ideal signals for macrophage scavenging functions.

The ability of MCP-1 to -4 and C5a to suppress IL-12 p70 production from monocytes, but not DC, is also consistent with a role for these factors in local environments, rather than in the priming of T cells in lymph nodes, a function normally ascribed to DC. In this sense, these factors may affect the production of IL-12 p70 from monocyte/macrophages and resulting IFN-γ production from NK cells as well as affect the survival of effector Th1 cells in inflamed tissues. Interestingly, the inability of MCP-1 to -4 and C5a to suppress IL-12 production from DC is unique, in that other IL-12 inhibitory signals, such as vitamin D_3, β_2-adrenergic agonists, and cholera toxin, affect both cell types equivalently (23, 24, 54). To begin to address the reasons why C5a does not effect DC production of IL-12, we analyzed the expression of the C5a receptor (CD88) on DC by flow cytometry. The lack of suppression clearly cannot be attributed to lack of receptor expression, as DC express the C5a receptor (Fig. 8). Interestingly, as determined by flow cytometry, the expression of CD88 by the less mature population of $CD1a^+$ DCs was bimodal, suggesting that there is heterogeneity of receptor expression by these cells.

Finally, the signaling pathway by which chemoattractants suppress IL-12 was explored. Signaling by chemoattractants is known to occur via seven-transmembrane domain receptors via the release of coupled G proteins from their heterotrimeric complexes. The fact that pertussis toxin reversed the inhibition of the chemoattractants (Fig. 6) demonstrates that the G_i is involved in the suppressive signals. Because activation of G_i is known to inhibit adenylate cyclase activity in many cell systems (reviewed in Ref. 55), this finding suggests that inhibition of IL-12 by chemoattractants is not dependent on the induction of high levels of intracellular cAMP. This finding is in contradistinction to the suppression of IL-12 by other factors that also signal through seven-transmembrane receptors such as PGE_2 (56) or β_2-adrenergic agonists (23). The inhibitory effects of these factors appear to be directly related to their ability to induce high levels of cAMP. Our findings are consistent, however, with what is known regarding the families of G proteins that couple to chemoattractant as opposed to other seven-transmembrane domain receptors, since G_s family members, which activate adenylate cyclase, do not appear to couple to chemoattractant receptors. What this implies is that the many factors that selectively suppress IL-12 production (15, 16, 19–24, 56–59), which act via disparate surface receptors and can induce discrete proximal intracellular signals, have a common downstream signaling pathway, the end result of which is the inhibition of IL-12 gene transcription.

In summary, our findings indicate that a select group of chemoattractants may be active participants in the development of T cell-mediated immune responses by acting to alter the balance of cytokines in the environment of differentiating T cells. They suggest that these chemoattractants may act as the ideal signals for scavenging functions of macrophages by functioning to attract and active these cells at the same time as preventing potentially harmful Th1 responses and toxic effects of IL-12. In conjunction with the previous finding of signaling via receptors for complement opsonins C3b or iC3b (19, 20, 22), the findings presented here with C5a contribute to the hypothesis that a reciprocal relationship exists between complement activation and the development of Th1 T cell responses. Finally, these studies have implications for understanding immunity to organisms that bind to chemokine receptors for their entry (60), produce chemokine analogues in the process of immune evasion (61), or generate active complement components, either directly or following opsonization with specific Ig.

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References

1. Trinchieri, G. 1998. Proinflammatory and immunoregulatory functions of interleukin-12. *Int. Rev. Immunol. 16:365.*
2. Trinchieri, G. 1998. Immunobiology of interleukin-12. *Immunol. Res. 17:269.*
3. Sieburth, D., E. W. Jabs, J. A. Warrington, X. Li, J. Lasota, S. LaForgia, K. Kelleher, K. Huebner, J. J. Wasmuth, and S. F. Wolf. 1992. Assignment of genes encoding a unique cytokine (IL-12) composed of two unrelated subunits to chromosomes 3 and 5. *Genomics 14:59.*
4. Snijders, A., C. M. Hilkens, T. C. van der Pouw Kraan, M. Engel, L. A. Aarden, and M. L. Kapsenberg. 1996. Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. *J. Immunol. 156:1207.*
5. Hayes, M. P., J. Wang, and M. A. Norcross. 1995. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-γ of lipopolysaccharide-inducible p35 and p40 genes. *Blood 86:646.*
6. Ma, X., J. M. Chow, G. Gri, G. Carra, F. Gerosa, S. F. Wolf, R. Dzialo, and G. Trinchieri. 1996. The interleukin 12 p40 gene promoter is primed by interferon γ in monocytic cells. *J. Exp. Med. 183:147.*
7. Cella, M., D. Scheidegger, L. K. Palmer, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med. 184:747.*
8. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10 [published erratum appears in 1996, *J. Exp. Med. 184:following 1590*]. *J. Exp. Med. 184:741.*
9. Stuber, E., W. Strober, and M. Neurath. 1996. Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J. Exp. Med. 183:693.*
10. Shu, U., M. Kiniwa, C. Y. Wu, C. Maliszewski, N. Vezzio, J. Hakimi, M. Gately, and G. Delespesse. 1995. Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. *Eur. J. Immunol. 25:1125.*
11. Kelsall, B. L., E. Stuber, M. Neurath, and W. Strober. 1996. Interleukin-12 production by dendritic cells: the role of CD40-CD40L interactions in Th1 T-cell responses. *Ann. NY Acad. Sci. 795:116.*
12. Hodge, D. J., P. W. Noble, M. R. Horton, C. Bao, M. Wysoka, M. D. Burdick, R. M. Strieter, G. Trinchieri, and E. Pure. 1997. Induction of IL-12 and chemokines by hyaluronan requires adhesion-dependent priming of resident but not elicited macrophages. *J. Immunol. 159:2492.*
13. Cassatella, M. A., L. Meda, S. Gasperini, A. D'Andrea, X. Ma, and G. Trinchieri. 1995. Interleukin-12 production by human polymorphonuclear leukocytes. *Eur. J. Immunol. 25:1.*
14. Kubin, M., J. M. Chow, and G. Trinchieri. 1994. Differential regulation of interleukin-12 (IL-12), tumor necrosis factor α, and IL-1β production in human myeloid leukemia cell lines and peripheral blood mononuclear cells. *Blood 83: 1847.*
15. D'Andrea, A., A. M. Aste, N. M. Valiante, X. Ma, M. Kubin, and G. Trinchieri. 1993. Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J. Exp. Med. 178:1041.*
16. D'Andrea, A., X. Ma, A. M. Aste, C. Paganin, and G. Trinchieri. 1995. Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor α production. *J. Exp. Med. 181:537.*
17. Belkaid, Y., B. Butcher, and D. L. Sacks. 1998. Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in *Leishmania*-infected cells. *Eur. J. Immunol. 28:1389.*
18. Carrera, L., R. T. Gazzinelli, R. Badolato, S. Hieny, W. Muller, R. Kuhn, and D. L. Sacks. 1996. *Leishmania* promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. *J. Exp. Med. 183:515.*
19. Karp, C. L., M. Wysocka, L. M. Wahl, J. M. Ahearn, P. J. Cuomo, B. Sherry, G. Trinchieri, and D. E. Griffin. 1996. Mechanism of suppression of cell-mediated immunity by measles virus [published erratum appears in 1997, *Science 275:1053*]. *Science 273:228.*
20. Sutterwala, F. S., G. J. Noel, R. Clynes, and D. M. Mosser. 1997. Selective suppression of interleukin-12 induction after macrophage receptor ligation. *J. Exp. Med. 185:1977.*
21. Berger, S., R. Chandra, H. Ballo, R. Hildenbrand, and H. J. Stutte. 1997. Immune complexes are potent inhibitors of interleukin-12 secretion by human monocytes. *Eur. J. Immunol. 27:2994.*
22. Marth, T., and B. L. Kelsall. 1997. Regulation of interleukin-12 by complement receptor 3 signaling. *J. Exp. Med. 185:1987.*
23. Panina, B. P., D. Mazzeo, P. D. Lucia, D. D'Ambrosio, R. Lang, L. Fabbri, C. Self, and F. Sinigaglia. 1997. β_2-Agonists prevent Th1 development by selective inhibition of interleukin 12. *J. Clin. Invest. 100:1513.*
24. D'Ambrosio, D., M. Cippitelli, M. G. Cocciolo, D. Mazzeo, L. P. Di, R. Lang, F. Sinigaglia, and B. P. Panina. 1998. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D_3: involvement of NF-κB downregulation in transcriptional repression of the p40 gene. *J. Clin. Invest. 101:252.*
25. Karpus, W. J., K. J. Kennedy, S. L. Kunkel, and N. W. Lukacs. 1998. Monocyte chemotactic protein 1 regulates oral tolerance induction by inhibition of T helper cell 1-related cytokines. *J. Exp. Med. 187:733.*
26. Bender, A., M. Sapp, G. Schuler, R. M. Steinman, and N. Bhardwaj. 1996. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J. Immunol. Methods 196:121.*
27. Gerard, C., and N. P. Gerard. 1994. C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu. Rev. Immunol. 12:775.*
28. Newton, R. C., and K. Vaddi. 1997. Biological responses to C-C chemokines. *Methods Enzymol. 287:174.*
29. Murphy, P. M. 1994. The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol. 12:593.*
30. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, K. C. Van, I. Durand, and J. Banchereau. 1994. Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med. 180:1263.*
31. Sallusto, F., P. Schaerli, P. Loetscher, C. Schaniel, D. Lenig, C. R. Mackay, S. Qin, and A. Lanzavecchia. 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol. 28:2760.*
32. Sozzani, S., F. Sallusto, W. Luini, D. Zhou, L. Piemonti, P. Allavena, J. Van Damme, S. Valitutti, A. Lanzavecchia, and A. Mantovani. 1995. Migration of dendritic cells in response to formyl peptides, C5a, and a distinct set of chemokines. *J. Immunol. 155:3292.*
33. Sozzani, S., W. Luini, A. Borsatti, N. Polentarutti, D. Zhou, L. Piemonti, G. D'Amico, C. A. Power, T. N. Wells, M. Gobbi, et al. 1997. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J. Immunol. 159:1993.*
34. Kalinski, P., J. H. Schuitemaker, C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-γ and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J. Immunol. 162:3231.*
35. Reiner, S. L., S. Zheng, Z. E. Wang, L. Stowring, and R. M. Locksley. 1994. *Leishmania* promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from $CD4^+$ T cells during initiation of infection. *J. Exp. Med. 179:447.*
36. Heath, H., S. X. Qin, P. Rao, L. J. Wu, G. LaRosa, N. Kassam, P. D. Ponath, and C. R. Mackay. 1997. Chemokine receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody. *J. Clin. Invest. 99:7.*
37. Ponath, P. D., S. X. Qin, T. W. Post, J. Wang, L. Wu, N. P. Gerard, W. Newman, C. Gerard, and C. R. Mackay. 1996. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J. Exp. Med. 183:12.*
38. Marth, T., W. Strober, and B. L. Kelsall. 1996. High dose oral tolerance in ovalbumin TCR-transgenic mice: systemic neutralization of IL-12 augments TGF-β secretion and T cell apoptosis. *J. Immunol. 157:2348.*
39. Neurath, M. F., I. Fuss, B. L. Kelsall, E. Stuber, and W. Strober. 1995. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J. Exp. Med. 182:1281.*
40. Jung, H. C., L. Eckmann, S. K. Yang, A. Panja, J. Fierer, W. E. Morzycka, and M. F. Kagnoff. 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J. Clin. Invest. 95:55.*
41. Yang, S. K., L. Eckmann, A. Panja, and M. F. Kagnoff. 1997. Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. *Gastroenterology 113:1214.*
42. Hornquist, C. E., X. Lu, F. P. Rogers, U. Rudolph, S. Shappell, L. Birnbaumer, and G. R. Harriman. 1997. G(α)i2-deficient mice with colitis exhibit a local increase in memory $CD4^+$ T cells and proinflammatory Th1-type cytokines. *J. Immunol. 158:1068.*

43. Whiteland, J. L., C. Shimeld, S. M. Nicholls, D. L. Easty, N. A. Williams, N. A. and T. J. Hill. 1997. Immunohistochemical detection of cytokines in paraffin-embedded mouse tissues. *J. Immunol. Methods 210:6.*
44. Braunstein, J., L. Qiao, F. Autschbach, G. Schurmann, and S. Meuer. 1997. T cells of the human intestinal lamina propria are high producers of interleukin-10. *Gut 41:6.*
45. Autschbach, F., J. Braunstein, B. Helmke, I. Zuna, G. Schurmann, Z. I. Niemir, R. Wallich, H. F. Otto, and S. C. Meuer. 1998. In situ expression of interleukin-10 in noninflamed human gut and in inflammatory bowel disease. *Am. J. Pathol.153:10.*
46. Berg, D. J., N. Davidson, R. Kuhn, W. Muller, S. Menon, G. Holland, L. Thompson-Snipes, M. W. Leach, and D. Rennick. 1996. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and $CD4^+$ TH1-like responses. *J. Clin. Invest. 98:11.*
47. Kuhn, R. J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell 75:12.*
48. Wysocka, M., M. Kubin, L. Q. Vieira, L. Ozmen, G. Garotta, P. Scott, and G. Trinchieri. 1995. Interleukin-12 is required for interferon-γ production and lethality in lipopolysaccharide-induced shock in mice. *Eur. J. Immunol. 25:672.*
49. Heinzel, F. P., R. M. Rerko, P. Ling, J. Hakimi, and D. S. Schoenhaut. 1994. Interleukin 12 is produced in vivo during endotoxemia and stimulates synthesis of γ interferon. *Infect. Immun. 62:4244.*
50. Zisman, D. A., S. L. Kunkel, R. M. Strieter, W. C. Tsai, K. Bucknell, J. Wilkowski, and T. J. Standiford. 1997. MCP-1 protects mice in lethal endotoxemia. *J. Clin. Invest. 99:5.*
51. Chensue, S. W., K. S. Warmington, J. H. Ruth, P. S. Sanghi, P. Lincoln, and S. L. Kunkel. 1996. Role of monocyte chemoattractant protein-1 (MCP-1) in Th1 (mycobacterial) and Th2 (schistosomal) antigen-induced granuloma formation: relationship to local inflammation, Th cell expression, and IL-12 production. *J. Immunol. 157:7.*
52. Wittmann, M., J. Zwirner, V. A. Larsson, K. Kirchhoff, G. Begemann, A. Kapp, O. Gotze, and T. Werfel. 1999. C5a suppresses the production of IL-12 by IFN-γ-primed and lipopolysaccharide-challenged human monocytes. *J. Immunol. 162:6763.*
53. Czermak, B. J., V. Sarma, C. L. Pierson, R. L. Warner, M. Huber-Lang, N. M. Bless, H. Schmal, H. P. Friedl, and P. A. Ward. 1999. Protective effects of C5a blockade in sepsis. *Nat. Med. 5:788.*
54. Braun, M. C., J. He, C. Y. Wu, and B. L. Kelsall. 1999. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor β1 and β2 chain expression. *J. Exp. Med. 189:541.*
55. Kehrl, J. H. 1998. Heterotrimeric G protein signaling: roles in immune function and fine- tuning by RGS proteins. *Immunity 8:1.*
56. van der Pouw Kraan, T. C., L. C. Boeije, R. J. Smeenk, J. Wijdenes, and L. A. Aarden. 1995. Prostaglandin-E_2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med. 181:775.*
57. Moller, D. R., M. Wysocka, B. M. Greenlee, X. Ma, L. Wahl, D. A. Flockhart, G. Trinchieri, and C. L. Karp. 1997. Inhibition of IL-12 production by thalidomide. *J. Immunol. 159:5157.*
58. Moller, D. R., M. Wysocka, B. M. Greenlee, X. Ma, L. Wahl, G. Trinchieri, and C. L. Karp. 1997. Inhibition of human interleukin-12 production by pentoxifylline. *Immunology 91:197.*
59. Sutterwala, F. S., G. J. Noel, P. Salgame, and D. M. Mosser. 1998. Reversal of proinflammatory responses by ligating the macrophage Fcγ receptor type I. *J. Exp. Med. 188:217.*
60. Pease, J. E., and P. M. Murphy. 1998. Microbial corruption of the chemokine system: an expanding paradigm. *Semin. Immunol. 10:169.*
61. Damon, I., P. M. Murphy, and B. Moss. 1998. Broad spectrum chemokine antagonistic activity of a human poxvirus chemokine homolog. *Proc. Natl. Acad. Sci. USA 95:6403.*