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Induction of Functional IL-8 Receptors by IL-4 and IL-13 in Human Monocytes

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IL-8 and related Glu-Leu-Arg (ELR⁺) CXC chemokines are potent chemoattractants for neutrophils but not for monocytes. IL-13 and IL-4 strongly increased CXCR1 and CXCR2 chemokine receptor expression in human monocytes, macrophages, and dendritic cells. The effect was receptor- and cell type-selective, in that CCRs were not increased and no augmentation was seen in neutrophils. The effect was rapid, starting at 4 h, and concentration dependent (EC₅₀ = 6.2 and 8.3 ng/ml for CXCR1 and CXCR2, respectively) and caused by new transcriptional activity. IL-13/IL-4-treated monocytes showed increased CXCR1 and CXCR2 membrane expression. IL-8 and related ELR⁺ chemokines were potent and effective chemotactic agents for IL-13/IL-4-treated monocytes, but not for untreated mononuclear phagocytes, with activity comparable to that of reference monocyte attractants, such as MCP-1. In the same cells, IL-8 also caused superoxide release. Macrophages and dendritic cells present in biopsies from Omenn’s syndrome and atopic dermatitis patients, two Th2 skewed pathologies, expressed IL-8 receptors by immunohistochemistry. These results show that IL-13 and IL-4 convert IL-8 and related ELR⁺ chemokines, prototypic neutrophil attractants, into monocyte chemotactic agonists, by up-regulating receptor expression. Therefore, IL-8 and related chemokines may contribute to the accumulation and positioning of mononuclear phagocytes in Th2-dominated responses. The Journal of Immunology, 2000, 164: 3862–3869.

Macrophages play a central role in immune and inflammatory responses and carry out a fundamental protective function against invading organisms (1–3). To accomplish this function, macrophages leave the blood compartment and accumulate at sites of inflammation and immune response. The inflammatory signals present locally are responsible both for recruitment, through the secondary induction of chemotactic factors, and activation of macrophages. Macrophages exposed to inflammatory agonists, such as endotoxin and IFN-γ, become activated with altered expression of surface Ags and receptors (e.g., Fcγ receptors and MHC class II molecules), increased production of proinflammatory cytokines (e.g., IL-1, IL-6, TNF, and chemokines), and enhanced capacity to produce reactive oxygen intermediates and kill intracellular pathogens (1–4). Alternatively, macrophages can be activated by IL-4 and IL-13, two Th2 cytokines, and these cells express a different activated profile consisting in the induction of the mannose receptor, MHC class II expression, IL-1 receptor antagonist, and type II IL-1 decoy receptor. On the contrary, alternative activated macrophages show reduced proinflammatory cytokine secretion (e.g., IL-1, TNF, IL-6, and chemokines) (2, 5–11). Although IFN-γ-activated macrophages resemble those found during the early phases of inflammation, alternative activated macrophages characterize chronic inflammatory diseases, psoriasis, and wound healing (2).

Chemokines play a central role in leukocyte extravasation and migration (12–17). CC (or β) chemokines (e.g., members of the monocyte chemotactic protein, and macrophage inflammatory protein clusters) are the main agonists for mononuclear leukocytes, including monocytes, lymphocytes, NK, and dendritic cells. Alternatively, members of the other major chemokine subfamily, the CXC (or α) chemokines (e.g., IL-8, growth-regulated oncogene (Gro) and IFN-γ-inducible protein-10 (IP-10)) are predominantly recognized as chemoattractants for neutrophils and lymphocytes (12–17). The major factor that dictates chemokine specificity for target cells is the regulated expression of chemokine receptors. Nine receptors for CC chemokines (CCR1–9) and five receptors for CXC chemokines (CXCR1–5) were cloned and characterized. Lymphotactin and fractalkine, the only representatives of two additional chemokine families, also bind specific receptors, XCR1 and CX3CR1, respectively (12–16, 18).

Inflammatory and immune signals are the major inducers of chemokine production both in vitro and in vivo. Endotoxins, IL-1, and

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3 Abbreviations used in this paper: Gro, growth-regulated oncogene; IP-10, IFN-γ-inducible protein-10; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; NAP, neutrophil activating protein; ELR, Glu-Leu-Arg.
TNF induce the production both of CC (e.g., monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3, and macrophage inflammatory protein-1α (MIP-1α)), and CXC (e.g., IL-8, neutrophil activating protein-2 (NAP-2), and Gro) chemokines by many cellular types, including mononuclear phagocytes and endothelial cells (12–15, 17). Of note, proinflammatory agonists also regulate the expression of chemokine receptors on leukocytes. LPS, TNF, and IFN-γ were shown to down-regulate the expression of CCR2, the receptor for MCP-1 to -4, in monocytes (19, 20) and CXCR1 and CXCR2, the two IL-8 receptors, in human neutrophils (21). Thus, the same signals generated during an inflammatory response have a reciprocal and opposite effect on chemokine and chemokine receptor expression on inflammatory cells, and this may represent an important mechanism to control chemokine specificity in vivo (12, 17).

The role of IL-4 and IL-13 on the chemokine system is more complex. Both cytokines were reported to inhibit the LPS-induced expression of MCP-1, MIP-1α, and IL-8 in monocytes, but were ineffective or indeed synergize the production of the same chemokines in endothelial cells (11, 12, 17). Furthermore, both IL-4 and IL-13 selectively induce the expression of certain chemokines, such as macrophage-derived chemokine (22, 23) and dendritic-cell-derived C-C chemokine-1 (DC-CK1/AMAC-1/PARC) in monocytes (2, 24, 25). The effect of these two Th2 cytokines on chemokine receptor expression in mononuclear phagocytes is presently unknown.

This work was undertaken to investigate whether IL-4 and IL-13, two cytokines that are able to induce a peculiar activation phenotype in mononuclear phagocytes, might also reorient the responsiveness of human monocytes by altering the expression of their chemokine receptor repertoire.

Materials and Methods

Cytokines

Human recombinant MCP-1 was from PeproTech (Rocky Hill, NJ). Human recombinant IL-8 was from Dainippon (Osaka, Japan). Human recombinant Gro-β and M-CSF were from Cetus (Emeryville, CA). Human recombinant GM-CSF was from Sandö (Milan, Italy). Human recombinant IL-8 was from Schering-Plough (Kenilworth, NJ). Human recombinant IL-4 was from Dainippon (Osaka, Japan). Human recombinant IL-13 was a kind gift from Dr. A. Minty (Sanofi Elf Bio Recherches, Labege, France). Cytokines were endotoxin free as assessed by Limulus amebocyte assay.

Cell preparation

Monocytes were obtained fromuffy coats of healthy blood donors through the courtesy of Centro Trasfusionale (Ospedale Sacco, Milan, Italy). Blood was washed once with saline and spun at 300 × g to remove plasma and platelets and then centrifuged on Ficoll (Biochrom, Berlin, Germany) at 95% monocytes (95% monocytes). These cells were >95% monocytes as evaluated by morphological analysis. PMN were purified by centrifugation on 46% iso-osmotic Percoll (Pharmacia, Uppsala, Sweden) gradient, as previously described (26). These cells were >95% neutrophils as evaluated by morphological analysis. Neutrophil viability in control and IL-4-treated cells was >80% at 24-h culture. Monocyte-derived dendritic cells and CD34+ cell-derived dendritic cells (CD34+–DC) were obtained by culturing in vitro monocyte and cord blood-purified CD34+ cells exactly as previously described (28). Macrophages were obtained incubating monocytes in petri dish cultures (Haereus, Vienna, Austria) for 7 days in RPMI with 10% FCS supplemented with 1000 U/ml M-CSF. Cells (5 × 10^6/ml) were stimulated with IL-4 and IL-13 in nonadhesion conditions in RPMI 1640 medium (Biochrom) with 10% FCS (HyClone, Logan, UT).

Northern blot analysis

Total RNA was extracted by the guanidinium thiocyanate method, blotted, and hybridized as described (26). Probes were labeled by Megaprime DNA labeling system (Amersham, Buckinghamshire, U.K.) with [α-32P]dCTP (3000 Ci/mmol, Amersham). Membranes were prehybridized at 42°C in Hybrisol (Onchor, Gaithersburg, MD) and hybridized overnight with 1 × 10^6 cpm/ml of [32P]-labeled probe. Membranes were then washed three times with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), at room temperature for 10 min, twice with 2× SSC, 1% SDS at 60°C for 20 min, and then with 0.1× SSC for 5 min, before being autoradiographed using Kodak (Rochester, NY) XAR-5 films and intensifier screens at −80°C. Specific CXCR1 probe was obtained with RT-PCR amplifying the region from 999 to 1341 bp of the reported sequence (GB M68932) with specific primers (5'-CTCAAGATCTGCTATGACATG-3' and 5'-GAATGATGGTGCTTCGTTCCATG-3'). Specific CXCR2 probe was obtained by amplifying the region 995-1415 bp of the reported sequence (GB M73969) with primers 5'-GGAATCCCTCAGATTCTAGCTATAC-3' and 5'-GTATGCGAGCTGTCTCAGG-3'. The extent of the hybridization was quantified by densitometric analysis with the entry level image system (Immagini e computer, Milan, Italy).

Migration assay

Cell migration was evaluated using a chemotaxis microchamber technique. Briefly, 27 μl of chemoattractant or control medium (RPMI 1640 with 1% FCS) were added to the lower wells of a chemotaxis chamber (Neuroprobe, Pleasanton, CA). Control medium 100 μl FCS were added to the upper chamber; then IL-4 (200 ng/ml) or IL-13 (200 ng/ml) were added to the lower wells of a chemotaxis chamber. The chamber was incubated at 37°C in humidified atmosphere in the presence of 5% CO2 for 90 min for monocytes and for 60 min for neutrophils. At the end of the incubation, filters were removed and stained, and five high power oil-immersion fields (×100) were counted. Results are expressed as the mean of three replicates ± SD of a single experiment representative of at least three independent donors.

Flow cytometric analysis of CXCR1 and CXCR2 expression

A specific (29) anti-CXCR1 mAb (5A12) was purchased from PharMingen (San Diego, CA). Anti-CXCR2 mAb (RII-115) was obtained as described (30). Monocytes were incubated with saturating amounts of mAbs and with fluorescein-conjugated F(ab')2 goat anti-mouse Ig (Techno Genetics, Turin, Italy). Analysis of fluorescence was performed by FACStarPLUS calibrated with Calibrate Beads (Becton Dickinson, Mountain View, CA).

Nuclear run-off

Nuclear run-off experiments were performed essentially as described (19, 26). Nuclei were isolated after 18 h of stimulation with IL-13 (20 ng/ml) or IL-4 (20 ng/ml). Then 60 μl of 5× run-off buffer (25 mM Tris–HCl, pH 8.2, 12.5 mM MgCl2, 750 mM KCl, and 1.25 mM each of ATP, CTP, and GDP) and 2 μl of RNase inhibitors 1 μl/μl (Perkin-Elmer-Cetus, Norwalk, CT), and 200 μCi of [α-32P]UTP 3000 Ci/ml (Amersham) were added to 220 μl of nuclei suspension and incubated at 30°C for 30 min. Elongated transcripts were then isolated with the guanidinium/cesium procedure and as described (19). RNA was denatured at 65°C for 5 min and hybridized at 42°C for 48 to 7 μg of denatured DNA immobilized on nitrocellulose filters in a few milliliters of hybridization solution (200 mM NaHPO4, pH 7.2, 1 mM EDTA, pH 8, 7% SDS, 45% deionized formamide, and 250 mg/ml yeast tRNA). Filters were then washed one or two times at 37°C for 20–30 min in 40 mM NaHPO4·1% SDS and exposed for autoradiography. Densitometric analysis was performed with the entry level image system (Immagini e computer), and fold increase was calculated after β-actin normalization.

Oxygen free radical production

Monocytes cultured for various time points in the presence or absence of IL-13 or IL-4 (20 ng/ml) were stimulated with 50 ng/ml PMA (Sigma, St. Louis, MO) or with chemokines (1 μg/ml). The production of H2O2 from NO was measured by dihydorhodamine 123 (DHR) oxidation, as previously described (31).

Electrophoresis and immunoblotting

Proteins were subjected to SDS-PAGE on 12% gels. Proteins were transferred to nitrocellulose membranes (Bio-Rad, Richmond, CA) as previously described (31). Blots were probed with rabbit anti-gp91phox, anti-p22phox, anti-p67phox, anti-p47phox, and anti-p40phox diluted 1:500 at 4°C overnight. Immunoreactive proteins were detected by enhanced chemiluminescence (ECL; Amersham). Multiple exposure of the same blot were performed to ascertain that the ECL signal was in the linear range of sensitivity.
Immunohistochemistry

IL-8 receptor expression was tested by immunohistochemistry on tissue samples from patients suffering from Omenn’s syndrome (two lymph nodes) and atopic dermatitis (one skin biopsy). Lymph node samples showing nonspecific reactive changes and normal skin biopsies were used as controls. Tissues were fresh frozen immediately after biopsy and stored at -80°C until used for immunostaining. mAb 5A12 was used at a dilution of 1:50, and immunoreactivity was developed using a Streptavidin-biotin immunoperoxidase technique, as described (32).

Results

The expression of chemokine receptors (CCR1 to CCR8, and CXCR1 to CXCR4) in human monocytes was first investigated by Northern blot analysis. By using this technique, no change in the basal expression profile of CC chemokine receptors was observed following IL-13 treatment (20 ng/ml for 24 h; data not shown). Consistently with previous results (33), the expression of CXCR1 and CXCR2 was barely detectable in resting monocytes. However, both IL-4 and IL-13 incubation strongly up-regulated the mRNA levels of the two IL-8 receptors (Fig. 1). CXCR3 expression was detectable neither in resting nor in IL-13-activated cells, whereas the expression of CXCR4 was slightly reduced in IL-13-activated cells (data not shown). The induction of CXCR1 and CXCR2 expression was clearly detectable after 4-h stimulation and became maximal at 24 h (Fig. 1A) with no further increase at 48 h (n = 2; data not shown). The effect of IL-13 was concentration dependent reaching a plateau at 20 ng/ml (data not shown), with EC50 values of 6.2 ± 1 ng/ml and 8.3 ± 0.3 ng/ml IL-13 for CXCR1 and CXCR2, respectively (n = 3; Fig. 1B). IL-4 paralleled the effect of IL-13 both in terms of concentration curve and kinetics (data not shown). The effect of IL-13 on CXCR1 and CXCR2 expression was at the transcriptional level, as assessed by nuclear run-off experiments. Resting monocytes showed a low rate of transcription of CXCR1 and CXCR2 mRNA. Transcription of both genes was strongly increased (~20-fold) by IL-13 treatment (Fig. 1C).

Changes in monocyte mRNA levels were paralleled by an increase in membrane expression of both CXCR1 and CXCR2 as assessed using specific mAbs by flow cytometry. In resting conditions, 8.3 ± 3% (range 0.5–23%) and 30.8 ± 8% (range 7–63%) monocytes expressed CXCR1 and CXCR2, respectively (n = 5). Both IL-13 and IL-4 strongly enhanced the expression of the two

FIGURE 1. Up-regulation of CXCR1 and CXCR2 by IL-13 and IL-4 in human monocytes. A and B, Northern blot analysis of human monocytes incubated for increasing times with 20 ng/ml IL-13 or IL-4 (A) or increasing concentrations of IL-13 for 24 h (B). Ten micrograms of total RNA were purified from monocytes and used in Northern blot analysis as described in Materials and Methods. Results are representative of at least three different donors. Ethidium bromide staining is shown in the lower part of the two panels. C, Run-off analysis. Human monocytes were incubated in the presence of 20 ng/ml IL-13 for 18 h. Equal loading of RNA probes were assessed by comparison to the β-actin gene. Result of one experiment, representative of three is shown.

FIGURE 2. Flow cytometry analysis of CXCR1 and CXCR2 expression by IL-13- and IL-4-stimulated human monocytes. Human monocytes were incubated with IL-13 or IL-4 (20 ng/ml) for 24 h. Cells were subsequently labeled with anti-CXCR1 (5A12), anti-CXCR2 (RII 115) or isotype-matched control mAbs followed by incubation with FITC goat anti-mouse Fab. Results of a single experiment, representative of at least four, are shown. Dotted and solid lines represents isotype control Ab and anti-IL-8 receptor Abs, respectively. Percentages of positive cells for CXCR1 and CXCR2 Abs and mean channel fluorescence (MCF) are reported.
IL-8 receptors (Fig. 2). At their optimal concentrations (20 ng/ml), the effect of the two cytokines was already detectable after 4-h incubation and reached maximal levels after 24 h with 4.2 ± 0.4- and 3.1 ± 0.9-fold of increase over control values for CXCR1 \((n = 4)\) and CXCR2 \((n = 5)\), respectively.

The functional relevance of the increased number of IL-8 receptors on IL-13-stimulated monocytes was investigated in terms of change in chemotaxis and in the release of oxygen radicals in response to IL-8. Fig. 3 shows that resting monocytes did not migrate in response to IL-8, a CXCR1 and CXCR2 ligand, and to Gro-β a CXCR2 selective ligand (Fig. 3, A and B). As expected, in the same experimental conditions, monocytes migrated in response to MCP-1 a chemokine active on monocytes (Fig. 3C). However, monocytes that had been exposed to IL-13 (20 ng/ml for 24 h) were able to respond to IL-8 in a chemotactic assay (Fig. 3A). Migration to IL-8 was concentration dependent with activity already present at 30 ng/ml (3.6 nM) and reaching a plateau between 100 and 1000 ng/ml (12–120 nM) IL-8. At the concentration of 300 ng/ml IL-8, the migration of monocytes was 62 ± 8% \((n = 9)\) of that observed in response to an optimal concentration of MCP-1 (50 ng/ml). Similar results were obtained when IL-4, instead of IL-13, was used (Fig. 3C and data not shown). Consistent with the kinetics observed by FACS analysis, migration to IL-8, and to Gro-β became detectable at 4-h stimulation with maximal activity observed at 24 h of IL-13/IL-4 stimulation (Fig. 3B and data not shown). Brief (30–60 min) exposure of monocytes to IL-13/IL-4 did not induce monocyte migration to IL-8 (data not shown). Basal migration and chemotaxis to MCP-1 were not modified by IL-13/IL-4 stimulation (Fig. 3C). Collectively, these results strongly suggest that the effect of IL-13 and IL-4 is not that of a general priming of monocyte migration but rather that it is mediated by up-regulation of specific IL-8 receptors.

IL-8 is known to induce release of oxygen radicals in human neutrophils (34, 35). Oxidative burst experiments were performed to assess whether IL-8 could also be active in inducing this response in IL-13/IL-4–stimulated monocytes. Fig. 4A shows that resting monocytes do not release superoxide anions in response to IL-8, but that this response was inducible in both IL-13- and IL-4-treated cells. In the early phase of the response (<5 min) the amount of IL-8–induced \(\text{H}_2\text{O}_2\) release was comparable to that induced by an optimal concentration of phorbol ester (PMA). However, at longer times of stimulation (>5 min) PMA action was stronger than IL-8, as expected (Fig. 4B). Gro-β (1 μg/ml) was also able to induce a respiratory burst in IL-4-treated monocytes,
although it was a weaker agonist than IL-8 (1.56 and 2.4 nmol/5 min/3 × 10⁶ cells for Gro-β and IL-8, respectively; n = 2). In the same experimental conditions IL-13/IL-4 did not modify the response of monocytes to PMA, or to MCP-1, a very weak activator of the oxidative burst in monocytes (36, 37). Short incubations with IL-13 (30 – 60 min) were not effective in inducing IL-8 response, and a 24-h incubation of monocytes with IL-13 did not change the protein levels of p40phox, p47phox, p67phox, p22phox, and gp91phox, five components of the NADPH oxidase enzyme in phagocytes (Fig. 4C) (38, 39). Again, collectively these results strongly suggest that IL-13 does not induce priming of monocytes for the activation of the oxidative burst, and that the response to IL-8 is mediated by the increased number of IL-8 receptors.

Since IL-13 has been shown to regulate certain biological responses in a cell-specific manner (40), the effect of IL-13 on CXCR1 and CXCR2 expression in different leukocyte subsets was investigated. Fig. 5 shows that similarly to monocytes IL-13 up-regulates the expression of IL-8 receptors in monocyte-derived macrophages. Dendritic cells generated in vitro from both monocytes and CD34⁺ precursors express basal levels of CXCR1 and CXCR2 (41, 42), and IL-13 is able to up-regulate this expression. Interestingly, IL-13 has an opposite effect on neutrophils, being able to down-regulate IL-8 receptor basal expression (~20-fold). This effect is functionally relevant, because PMN incubated with 20 ng/ml IL-13 show a significant inhibition in their chemotactic response to IL-8 (47% inhibition with 100 ng/ml IL-8; n = 2).

Finally, to gain insight into the possible in vivo relevance of these results, the expression of IL-8 receptors was investigated in two Th2-skewed pathologies characterized by the presence of IL-4 and IL-13, namely the Omenn’s syndrome and atopic dermatitis (43, 44). As expected, on the basis of the observations obtained in vitro, in normal tissues IL-8 receptor expression was detected only on neutrophils, whereas various monocyte-derived cells (e.g., tingible-body macrophages of germinal centers; interstitial macrophages) and dendritic cells (e.g., interdigitating dendritic cells and Langerhans cells) resulted completely negative (Fig. 6, A and B). On the contrary, in Omenn’s syndrome the macrophages and dendritic cells that typically accumulate within lymph nodes (43) showed strong reactivity for CXCR1 mAb (Fig. 6C). Similarly, in atopic dermatitis several CXCR1 immunoreactive mononuclear

![FIGURE 5. Regulation of IL-8 receptor expression by IL-13 in leukocytes. CXCR1 and CXCR2 expression was evaluated by Northern blot analysis in human monocytes, monocyte-derived macrophages, monocyte-derived dendritic cells (mono-DC), CD34⁺ cell-derived dendritic cells (CD34⁺-DC), and neutrophils (PMN) incubated in the presence or absence of 20 ng/ml IL-13 for 24 h. Ten micrograms of total RNA were purified and used in Northern blot analysis as described in Materials and Methods. Results are representative of two different donors.](http://www.jimmunol.org/)

![FIGURE 6. Immunohistochemistry for IL-8 receptor expression in vivo. One lymph node with nonspecific reactive changes (A and B), one lymph node with Omenn’s syndrome (C), and one skin biopsy from atopic dermatitis (D–F) were investigated for their immunoreactivity with the 5A12 mAb. A and B. No immunoreactivity for IL-8 receptors in the lymphoid cells and macrophages populating the lymph node are shown; only intravascular neutrophils are stained (B). Several macrophages and dendritic cells are labeled by anti-IL-8 receptor in the case of Omenn’s syndrome (C). In the skin with atopic dermatitis, IL-8 receptor positive cells are represented by dermal mononuclear cells (D), monocyte-derived cells forming intraepidermal collections (E), and intraepidermal dendritic cells (F). Immunoreactivity was detected by immunoperoxidase technique and light counterstain with Mayer’s haematoxylin. Magnifications shown are as follows: ×160 (A) and ×400 (B–F).](http://www.jimmunol.org/)

UP-REGULATION OF IL-8 RECEPTORS BY IL-4 AND IL-13
cells were detected in the dermis (Fig. 6D). In addition, collections of mononuclear cells within the epidermis, immunophenotypically identified as monocyte-derived cells (CD11b+/Mac1, CD11c+, CD36+, CD68+) or as immature dendritic cells (CD1a+) (data not shown) were also recognizable (Fig. 6E). Finally, anti-IL8 receptor Abs strongly labeled CD1a+ (data not shown) intraepidermal cells with obvious dendritic morphology (Fig. 6F). Unfortunately, neither of the two anti-CXCR2 Abs tested (RIII115 and 6C6) reacted in tissue sections, precluding a comparative analysis of CXCR2 expression in these two pathologies (data not shown).

Discussion

This study shows that IL-13 and IL-4, two prototypic Th2 cytokines that share many anti-inflammatory effects, up-regulate the expression of the two IL-8 receptors, CXCR1 and CXCR2, in human monocytes and in related leukocytes, such as macrophages and dendritic cells, in a time- (t1/2 = 15 and 12 h, respectively) and concentration- (EC50 = 6.2 and 8.3 ng/ml for CXCR1 and CXCR2, respectively) dependent fashion. CXCR1 and CXCR2 membrane expression on IL-13-treated monocytes increased about 4- and 3-fold, respectively. Increased expression of the two IL-8 receptors was paralleled by an acquired responsiveness of monocytes to IL-8 and Gro-α, a related CXC chemokine. IL-8 was able to induce directional migration and activation of the respiratory burst in IL-13/IL-4-treated monocytes in a range of concentrations similar to that active on PMN (34, 35). In these experimental conditions, IL-13/IL-4 did not induce a general priming of monocytes but specifically induced functional IL-8 receptors. This conclusion is based on the following observations: 1) the effect of the two cytokines required at least 4 h incubation and it was not observed at shorter (e.g., minutes) incubation times generally required for priming (45–47); 2) the kinetics of monocyte response to IL-8 closely paralleled the induction of membrane CXCR1 and CXCR2 expression; 3) IL-13/IL-4 treatment was specific for IL-8 and Gro-α with no effect on other monocyte agonists (i.e., MCP-1, and PMA); and 4) IL-13/IL-4-treated monocytes did not show changes in the expression of NADPH oxidase enzyme components.

IL-8 is part of a subset of CXC chemokines that share a tripeptide motif (i.e., Glu-Leu-Arg, ELR) at the NH2 terminus. This group includes, in addition to IL-8, Gro (α/β/γ), granulocyte chemotactic protein-2 (GCP-2), epithelial neutrophil-activating protein-78, NAP-2, and platelet factor-4 (14, 16, 48). IL-8 and GCP-2 activate target cells through the interaction with both CXCR1 and CXCR2, whereas all the other members of the group use only CXCR2 (14, 16, 48). Although CXCR1 and CXCR2 have been described to be expressed by a number of different leukocyte subsets (e.g., neutrophils, T and B lymphocytes, NK cells, mast cells, and eosinophils), ELR-CXC chemokines are considered to be relevant chemotactic signals only for granulocytes (14, 16, 17, 48). Human monocytes express low levels of surface IL-8 receptors (49–51), with CXCR2 being more expressed than CXCR1 (Ref. 50 and Fig. 2). These receptors can weakly flux calcium and trigger a weak respiratory burst in Con A-primed monocytes (47). Nevertheless, IL-8 cannot be considered an activator of monocyte functions (14, 16, 17, 48, 52, 53). The observations reported here indicate that freshly isolated human monocytes do not respond to IL-8 in terms of chemotaxis and release of oxygen radicals, but that these responses can be readily induced following exposure to IL-4 and IL-13 and subsequent up-regulation of CXCR1 and CXCR2. Regulation of chemokine receptor expression by pro- and anti-inflammatory signals has recently emerged as a key setpoint for chemokine action (19–21, 26, 54, 55). Specifically, CXCR1 and CXCR2 expression were shown to be up-regulated by G-CSF and down-regulated by LPS, GM-CSF, and TNF-α in neutrophils (21, 56). However, different from all the previous reports, the effect of IL-13 on CXCR1 and CXCR2 was at the level of gene transcription rather than on mRNA stability (19, 20, 26, 54, 57, 58). Very recently it was reported that IL-8 can induce firm adhesion of monocytes to vascular endothelium under flow conditions (59), and that CXCR2 may mediate the accumulation of macrophages in atherosclerotic lesions of low density lipoprotein receptor-deficient mice (60). The present study extends these observations and supports a role for ELR-chemokines in the recruitment and activation of mononuclear phagocytes in pathological conditions in which IL-4 and IL-13 are expressed, such as Th2 immune responses (61).

This hypothesis is supported by the demonstration that monocyte-derived cells and dendritic cells express IL-8 receptors in Omenn’s syndrome and atopic dermatitis, two pathological conditions typically characterized by a Th2-dominated immune response and IL-4 production (43, 44, 61).

IL-8 has been shown to be an important mediator of chronic inflammatory diseases like allergic bronchial asthma, rheumatoid arthritis, and psoriasis (62–64). In vivo, neutrophils predominate at early stages of inflammation, whereas monocytes and lymphocytes characterize late inflammatory phase reactions and chronic inflammation (65). Interestingly, we found that IL-4 and IL-13 down-regulate CXCR1 and CXCR2 expression in neutrophils and reduce their ability to migrate in response to IL-8. This evidence is confirmed in vivo in a mouse model of acute lung injury in which exogenously administered IL-13 reduces neutrophil counts in bronchoalveolar fluids, whereas an anti-IL-13 Ab has an opposite effect in mice administered IgG immune complexes (66). IL-13 down-regulates the expression of ICAM-1 in vascular endothelium (40, 67). ICAM-1/β2 integrin interaction is a crucial event for neutrophil endothelial cell transmigration in vitro and neutrophil recruitment in vivo (68, 69). On the contrary, IL-13 up-regulates the expression of VCAM-1, the counterreceptor of very late Ag-4, a β1 integrin expressed by monocytes and eosinophils and relevant for their interaction with cytokine-activated endothelium (67, 70, 71). IL-13 and IL-4 can act in synergism with TNF to promote the secretion of IL-8 by human endothelial cells (67, 72, 73). Collectively, it is tempting to speculate that IL-13 and IL-4 can trigger a biological program that reorients the action of IL-8 from neutrophils to monocytes and contributes to the formation of the mononuclear phagocyte infiltrate that characterize chronic inflammatory lesions. In this respect it is interesting to note that IL-8 is expressed by lesional macrophage-derived foam cells (74), and KC/Gro is present in atherosclerotic lesions (60).

The leukocyte infiltrate of IL-13-secreting tumors has a major mononuclear phagocyte component (75). It is tempting to speculate that IL-13 might act directly in the recruitment of monocytes for its chemotactic activity (76), or by inducing the expression of IL-8 receptors on these effector cells.

IL-8 and related CXC chemokines have long been known to be selective neutrophil attractants in vitro and in vivo (53, 77, 78), with little or no effect on monocytes. The results presented in this paper show that IL-4 and IL-13 convert ELR+ CXC chemokines into potent monocyte attractants by up regulating receptor expression. Furthermore, this study reports that two Th2 pathologies characterized by the presence of IL-4 and IL-13 are characterized by an infiltrate of IL-8 receptor positive mononuclear cells. Therefore, IL-8 and other CXC chemokines, whose production is weakly induced in endothelial cells by IL-4 and IL-13, may contribute to the accumulation and positioning of alternatively activated macrophages in Th2-dominated responses (2, 61).
References
