Autocrine regulation of interleukin-8 production in human monocytes

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Chemokines bind and activate lymphocytes, monocytes, macrophages, and polymorphonuclear neutrophils (PMNs). In addition to their primary function of attracting leukocytes to the sites of inflammation, chemokines may affect biological processes such as the development of stem cells, angiogenesis, and virus entry into host cells (reviewed in Ref. 1).

Interleukin (IL)-8 is one of the best-characterized C-X-C chemokines, the potent chemotactic activity of which has been associated with numerous acute and chronic inflammatory disorders (1). Two receptors for IL-8 have been characterized and named CXCR1 (IL-8 receptor type A) and CXCR2 (IL-8 receptor type B) (10, 19, 23). CXCR1 is specific for IL-8, whereas CXCR2 exhibits typical chemokine promiscuity in agonist recognition, being able to bind not only IL-8 but also neutrophil-activating peptide-2, melanoma growth-stimulatory activity/growth-related protein-α (MGSA/GROα), and 78-amino acid endothelial cell-derived neutrophil activator (26). IL-8 receptors are widely expressed in PMNs, peripheral blood mononuclear cells (MNCs), T lymphocytes, and natural killer cells. In addition, IL-8 receptors are also found in endothelial cells, neuronal cells, fibroblasts, and keratinocytes. Accordingly, IL-8 has been shown to possess functions such as the regulation of growth (4, 24) and angiogenesis (15). In leukocytes, IL-8 has been shown to stimulate the activation of G proteins and several downstream serine/threonine kinases (14, 16) that are responsible for chemotaxis, degranulation, and production of superoxide anions by phagocytes.

Because IL-8 plays a critical role in acute inflammation, it is important to understand how IL-8 expression is regulated. IL-8 production at the sites of inflammation requires de novo biosynthesis (1, 22). The IL-8 gene promoter contains sites for nuclear factor (NF)-κB, NF-IL-6, and activator protein-1 (22), transcription factors that regulate the expression of a large number of proinflammatory cytokines and growth factors. Accordingly, IL-8 production in various cell types can be readily induced by lipopolysaccharide, IL-1β, and tumor necrosis factor (TNF)-α. Chemokine receptors also

Chemoattractants produced at sites of inflammation are critical for the attraction and activation of leukocytes. Two major classes of leukocyte chemoattractants have been identified. The classic chemoattractants, including C5a, C3a, formyl-Met-Leu-Phe (fMLP), platelet-activating factor, and leukotriene B4, are potent activators of many phagocyte functions, although their chemical structures vary considerably. In contrast, the chemotactic cytokines (chemokines) are a group of peptides with a molecular mass of 8–10 kDa that share a structural homology including the positioning of cysteine residues (e.g., C-X-C vs. C-C) (1, 25).

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have varying potencies as inducers of IL-8 production (5). Recent findings (30) suggested that chemotactic receptors, like a number of other G protein-coupled receptors, mediate the activation of NF-κB and activator protein-1 and actively regulate the expression of genes that are controlled by these transcription factors. We have shown that both fMLP and C5a are able to stimulate the expression of IL-8 in monocytes and macrophages (3, 11). Like these chemotactants, IL-8 activates a signaling pathway that involves G protein activation and therefore can possibly lead to transcription activation. In this study, we sought to identify the role of IL-8 as an autocrine regulator of IL-8 production in peripheral blood leukocytes.

**MATERIALS AND METHODS**

**Reagents.** The chemokines were purchased from PeproTech (Rocky Hill, NJ); and fMLP, cycloheximide, and actinomycin D were from Sigma (St. Louis, MO). Indo-1 AM was from Molecular Probes (Eugene, OR). Mouse TNF-α was a gift from Dr. V. Kravchenko ( Scripps Research Institute, La Jolla, CA). The reagents used for nuclear runoff assay, including RNase-free DNase I, RNase A, yeast tRNA, and salmon sperm DNA, were from Boehringer Mannheim (Indianapolis, IN). Unless otherwise indicated, all other reagents were purchased from Sigma.

**Preparation of cells.** Human peripheral blood leukocytes were fractionated with Percoll as previously described (3). Briefly, blood was collected from healthy donors, with acid citrate-dextrose (citrate buffer containing 2% dextrose) as an anticoagulant. Erythrocytes were removed by sedimentation with HESPA (6% hetastarch; Baxter, Highland, CA). MNCs and PMNs in the supernatant were further separated by centrifugation at 450 g for 40 min at 10°C through Percoll step gradients (70 and 55%). Viability of the cells in a routine preparation was ~98% as determined by trypan blue exclusion. In some cases, monocytes and lymphocytes were further separated from preparations of MNCs. Briefly, MNCs were washed four times in PBS containing 1 mM EDTA and then resuspended in serum-free RPMI 1640 medium at a density of 10^6 cells/ml. The cells were stimulated with the appropriate ligands for 120 min, after which the cells and beads were removed from the medium by centrifugation and the supernatant was transferred to a new tube and stored at −80°C. Measurement of IL-8 secreted into the medium was performed by ELISA (BioSource, Camarillo, CA) according to the manufacturer’s instructions.

**Ca^{2+} mobilization.** The cells were loaded with indo 1-AM (5 μM) in Hanks’ balanced salt solution. Intracellular Ca^{2+} mobilization experiments were conducted with cells in suspension and were monitored by continuous fluorescent measurements in an SLM 8000 photon-counting spectrofluorometer (SLM-Aminco, Urbana, IL), with an excitation wavelength of 340 nm and detection at 400 and 490 nm. Relative intracellular Ca^{2+} level is expressed as the ratio of F/F_{min} determined at 400 nm to that at 490 nm. Intracellular free Ca^{2+} concentration ([Ca^{2+}_{i}]) was determined with the formula [Ca^{2+}_{i}]=2500F_{min}/(F_{max}−F), where F is the ratio of fluorescence obtained after ligand stimulation, F_{max} is the ratio of fluorescence obtained with Triton X-100 (0.1%) and reflects the total available free Ca^{2+}, and F_{min} is the ratio of fluorescence obtained with EDTA (2 mM) included in the assay buffer to remove free Ca^{2+} released by Triton X-100 treatment.

**Measurement of IL-8 transcripts.** Freshly isolated cells were stimulated with the appropriate ligands (100 nM each IL-8 and MGSA/GROα or 40 ng/ml of TNF-α) at 5 × 10^6 cells/ml in a total volume of 10 ml. After 2 h, the cells were harvested, and total RNA was extracted with the guanidinium thiocyanate method. cDNA was prepared with Superscript reverse transcriptase (GIBCO BRL, Life Technologies, Gaithersburg, MD). Amplification of IL-8 transcripts was accomplished with primers located within the coding region of IL-8 with the following sequences: forward primer, 5'-TGACTTCCAAGCTTGCCGTG-3' and reverse primer, 5'-ACAGAGCTCTCCTCCATCAG-3'. PCR amplification was conducted for 18 cycles to avoid overamplification. Equal amounts of the PCR products were analyzed on 1% agarose gels by electrophoresis and ethidium bromide staining. The relative intensity of the IL-8 message in each sample was determined with ImageQuant software (Molecular Dynamics, Mountain View, CA) and standardized against the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplified in the same tube.

**Nuclear runoff.** Nuclear transcripts of IL-8 were measured as follows. Briefly, 3 × 10^7 freshly isolated MNCs were incubated for 2 h with 100 nM IL-8 or MGSA/GROα or without chemokines (basal) in 4 ml of RPMI 1640 medium at 37°C. Nuclear suspensions were prepared by washing the cells with PBS followed by resuspension in 4 ml of lysis buffer (10 mM Tris·HCl, pH 7.4, 3 mM MgCl₂, 10 mM NaCl, and 0.5% Nonidet P-40) added dropwise with continuous vortexing followed by centrifugation at 500 g for 5 min. Nuclear pellets were washed one more time in 4 ml of lysis buffer and then maintained at −80°C in 230 μl of freezing buffer (50 mM Tris·HCl, pH 8.3, 40% glycerol, 5 mM MgCl₂, and 0.1 mM EDTA, pH 8.0) until used. The nuclear transcripts were elongated by incubating the nuclei with 60 μl of 5 × reaction buffer (150 mM Tris·HCl, pH 8.0, 750 mM KCl, 25 mM MgCl₂, 1 mM EDTA, 12.5 mM dithiothreitol, 2.5 mM each ATP, GTP, and CTP, and 100 μCi of [³²P]UTP) for 30 min at 30°C. Labeled nuclei were treated with 5 U of DNase I for 5
min at 30°C and then with 50 μg of proteinase K for 30 min at 37°C. Before isolation of the labeled transcripts, 200 μg of yeast tRNA were added as a carrier, and RNA was prepared with the guanidinium isothiocyanate method with the TRI Reagent (Molecular Research Center, Cincinnati, OH). RNA was denatured with 0.1 N NaOH on ice for 10 min followed by neutralization with 1 M HEPES, pH 7.4. RNA was precipitated by ethanol and resuspended in 1 ml of hybridization solution (see below) before use. For preparation of the filters, 1 μg of circular DNA was denatured by boiling in 0.1 N NaOH for 6 min followed by neutralization with HEPES buffer (pH 7.4) and dilution with 6× saline-sodium citrate (SSC) buffer before application to the filter with a slot blot apparatus. Filters were rinsed briefly in 6× SSC and baked for 2 h at 80°C. The filters were prehybridized [10 mM N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), 2% SDS, 10 mM EDTA, and 300 mM NaCl] for 2 h at 60°C followed by hybridization in the same solution with labeled RNA probe for an additional 36 h. Filters were washed twice for 15 min each in 2× SSC at 60°C followed by incubation for 30 min at 37°C in 2× SSC containing 1 μg/ml of RNase A. After an additional wash at room temperature in 2× SSC, the filters were air-dried and exposed to a phosphor-imaging screen.

Cell staining and flow cytometry. Fluorescence-activated cell analysis was performed on a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA). To identify monocytes in mononuclear fractions of human blood, cells were labeled with FITC-conjugated anti-CD14 monoclonal antibody (MAb; PharMingen, San Diego CA). Identification of the levels of each type of IL-8 receptor was done by incubating MNCs with phycoerythrin-conjugated MAb (1:500; PharMingen) directed against CXCR1 or CXCR2. All incubations were carried out on ice for 60 min. The cells were washed twice with PBS before flow cytometry analysis.

Detection of phosphorylated extracellular signal-regulated kinase. Freshly isolated MNCs (5 × 10⁶ cells in 250 μl of RPMI 1640 medium) were stimulated with either 100 nM IL-8 or 100 nM MGSA/GROα. Samples were harvested at different time points over a period of 15 min. Stimulation was stopped by adding 1 ml of ice-cold PBS followed by centrifugation. The cell pellets were resuspended in SDS-PAGE sample buffer and immediately boiled for 5 min. The extracts were then separated on 10% polyacrylamide gels and blotted to nitrocellulose filters. Filters were treated for 20 min with PBS containing 0.05% Tween 20 and 4% BSA and then incubated with anti-phospho-extracellular signal-regulated kinase (ERK) 1/2 (New England Biolabs, Beverly, MA). The bands were then stained with a peroxidase-conjugated goat secondary antibody (Ab) followed by visualization with chemiluminescence (Pierce, Rockford, IL).

RESULTS

IL-8 stimulates IL-8 production through an autocrine loop. A previous study (30) showing IL-8 induction of αB binding activity suggested that IL-8 may serve as an autocrine regulator of IL-8 production. This possibility and the underlying mechanism were investigated in the present study. A technical difficulty associated with this endeavor is the separation of newly synthesized IL-8 from the added ligand. Our initial efforts involved metabolically radiolabeling cells with [35S]methionine or [3H]leucine followed by immunoprecipitation of newly synthesized IL-8 from the culture medium. This method produced results that varied considerably due to interference of the added ligand with immunoprecipitation of the radiolabeled IL-8. To overcome this problem, IL-8 protein was conjugated to Affi-Gel (Sepharose) beads, and the immobilized IL-8 was then used as an agonist. The conjugated beads contain IL-8 at a concentration of ~70 nM in a 50% slurry. Moreover, incubation of bead-immobilized 125I-IL-8 with blood leukocytes for 2 h caused no detectable release of radiolabeled IL-8. Therefore, it is feasible to detect newly synthesized IL-8 in the culture medium after removal of the agonist (IL-8-conjugated beads) by centrifugation. Using this approach, we found that the immobilized IL-8 induced a sevenfold increase in IL-8 production from MNCs over a 2-h period (Fig. 1A). Further incubation resulted in additional increase in IL-8 secretion at 4 h (Fig. 1B). To minimize the possible secondary effects on IL-8 biosynthesis, incubation time was limited to 2 h in subsequent experiments.

The induced IL-8 production by IL-8-conjugated beads was determined to be specific to IL-8 because...
beads conjugated in a similar fashion with BSA or MGSA/GROα did not initiate secretion of IL-8 into the medium (Fig. 1). Unconjugated MGSA/GROα and BSA also failed to stimulate IL-8 synthesis (data not shown). These findings suggest that stimulation of IL-8 production is a property of IL-8 but not of Affi-Gel beads.

**Autocrine production of IL-8 is cell-type specific.** The distribution of the two IL-8 receptors in different leukocytes has been reported by several groups (9, 18, 20) with slightly different results. Using specific MAbs against CXCR1 and CXCR2, we observed that PMNs express more CXCR1 and CXCR2 than either monocytes or lymphocytes (Fig. 2A). Also in agreement with the previous findings, donor variation was evident, but in each preparation, monocytes always expressed relatively low levels of both receptors, and CXCR2 was the more abundant of the two subtypes. The presence of small amounts of both CXCR1 and CXCR2 in a lymphocyte subpopulation, probably natural killer cells (27), necessitated investigation of the source of IL-8 secretion in the MNC preparations. By enrichment of monocytes from the MNC fractions to ~85%, it was determined that the immobilized IL-8 could stimulate almost twice as much IL-8 secretion in monocytes as in unenriched MNCs (Fig. 2B). Furthermore, the enriched lymphocyte population (~85%) responded with less than half as much secreted IL-8 as with MNCs (Fig. 2B). These data indicate that monocytes within the MNC preparations are the principal cells responding to the immobilized ligand with production of IL-8.

IL-8 and MGSA/GROα can activate PMNs (1). Browning et al. (3) and Cassatella (5) have previously shown that PMNs responded to fMLP stimulation with an increase in IL-8 production. However, PMNs were unresponsive in this capacity to both the immobilized IL-8 and MGSA/GROα (Fig. 1A). Because PMNs expressed more IL-8 receptors than monocytes but did not respond with IL-8 production, it was important to demonstrate that the prepared neutrophils were viable and responsive to these ligands. With Ca2+ mobilization as an indicator, it was determined that the PMN preparations were able to respond to both IL-8 and MGSA/GROα by increasing free [Ca2+]i (Fig. 2C). Furthermore, the magnitude of the Ca2+ response to IL-8 and MGSA/GROα was similar to that induced by fMLP. Therefore, the lack of IL-8 synthesis in IL-8-stimulated PMNs is a cell-specific phenomenon, not related to cell surface expression of the receptors and Ca2+ mobilization. This result also indicates that induction of the Ca2+ response is not sufficient for IL-8 production in neutrophils, which may require additional signaling events.

**The role of transcription and translation in autocrine IL-8 production.** It has recently been shown that several chemoattractants including leukotriene B4, platelet-activating factor, and fMLP can activate transcription in peripheral blood monocytes (2, 3, 11, 30). To determine whether the secreted IL-8 was the result of de novo synthesis, the effect of transcription and trans-
diation inhibitors (actinomycin D and cycloheximide, respectively) on the production of IL-8 was investigated. Preincubation of MNCs for 1 h with increasing doses of these inhibitors followed by stimulation of the cells for 2 additional hours with the immobilized IL-8 resulted in a dose-dependent inhibition of the IL-8 detected in the medium (Fig. 3A). The dose-response curves for both actinomycin D and cycloheximide were similar, suggesting the importance of both transcription and translation for the production of IL-8 in these experiments. The inhibition of IL-8 production was significant (≈60% by 1 h pretreatment), suggesting de novo synthesis of IL-8 in response to IL-8 stimulation.

Additional experiments with RT-PCR were conducted to estimate the effect of IL-8 on the accumulation of IL-8 transcript in MNCs. mRNA for IL-8 was detected in the unstimulated MNCs, but the relative amount increased markedly in response to IL-8 (Fig. 3B). TNF-α was similarly able to increase the level of IL-8 message relative to the basal level. With this method, it was found that MGSA/GROα was unable to increase the IL-8 mRNA over the baseline level in MNCs, consistent with the lack of IL-8 accumulation in the culture medium (Fig. 1A). Because the above procedure detects only cytosolic mRNA levels that can be affected by other posttranscriptional factors, we sought to examine the levels of nuclear IL-8 transcript in runoff experiments (Fig. 3C). Similar to results obtained with RT-PCR, there was a low level of IL-8 transcript in unstimulated cells (relative to GAPDH). The nuclear IL-8 transcript level was increased markedly when stimulated with IL-8 but only marginally with MGSA/GROα. Neither IL-8 nor MGSA/GROα were able to increase the transcription of the MGSA/GROα gene as shown by the nuclear runoff experiment (Fig. 3C).

Stimulation of MNCs with increasing amounts of IL-8-conjugated beads resulted in a dose-dependent increase in the IL-8 secreted into the medium (Fig. 4A). The dose-response curve followed classic receptor-mediated kinetics, with an apparent ED50 of 1.4 nM (20 nM IL-8 beads) and a saturation point of ≈3.5–4.0 nM (≈50 µl of IL-8 beads) based on quantitation of 125I-labeled IL-8 attached to the beads. The concentration of IL-8 detected in the medium after 2 h at a saturating dose of IL-8 beads approached 0.25 nM when 1 × 10^6 cells were used. This value continued to increase with a longer incubation time or higher cell density, although higher background secretion was noted (data not shown). At a concentration of up to 2 orders of magnitude above the dissociation constant for CXCR2, immobilized MGSA/GROα did not illicit an IL-8 response in MNCs (Fig. 4A). This result was unexpected because CXCR2 is more abundant than CXCR1 in monocytes. To further explore the role of CXCR2 in the induction of IL-8 secretion, MNCs were incubated with 200 nM MGSA/GROα for 20 min before the addition of immobilized IL-8. MGSA/GROα pretreatment has been reported to result in desensitization and partial downregulation of CXCR2 responsiveness (7, 21). However, this treatment of MNCs with MGSA/GROα had no detectable effect on the responsiveness of the cells to subsequent stimulation with the IL-8 beads (Fig. 4B), indicating a lack of desensitization by MGSA/GROα. Because MGSA/GROα binds to CXCR2 but not to CXCR1, these data combined suggest preferential usage of CXCR1 in IL-8-stimulated IL-8 production.

**Fig. 3.** Involvement of de novo RNA and protein synthesis in IL-8-induced secretion of IL-8. A: freshly isolated MNCs (1 × 10^6 cells in 1 ml) were preincubated with indicated concentrations of actinomycin D (ActD) or cycloheximide (CHX) for 1 h. Cells were then stimulated with 20 µl of IL-8-conjugated Sepharose beads (equivalent to 1.4 nM IL-8) for 2 h. The IL-8 secreted into the medium was measured by ELISA. Data are means ± SE of 2 independent experiments. B: changes in IL-8 message level were measured in unstimulated MNCs (basal), MNCs stimulated with IL-8, MGSA, or tumor necrosis factor (TNF-α) as described in MATERIALS AND METHODS. Each sample contained 5 × 10^6 cells. Stimulation was carried out for 2 h, and cytosolic IL-8 transcript was determined by RT-PCR. The expression levels of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined in the same PCR and used for standardization. Data presented are from 1 of 2 experiments with similar results. C: nuclear runoff analysis was used to examine the effects of IL-8 and MGSA on the levels of transcription of the genes for IL-8 and MGSA as described in MATERIALS AND METHODS. Each sample contained 3 × 10^7 MNCs. Equal loading of RNA probe was assessed by comparison with the housekeeping gene GAPDH.
Involvement of mitogen-activated protein kinase activation in IL-8 secretion by MNCs. Mitogen-activated protein (MAP) kinase cascades are important mediators of transcription factor activation leading to gene expression in response to a variety of cellular stimuli. The most widely studied MAP kinase pathway is activated upstream by the small GTPase Ras, which leads to activation of Raf and MAP kinase kinase kinase (MEK) 1/2 and ultimately to the phosphorylation of ERK. Activation of ERK has been linked to IL-8 production in a previous study (6) with the lung epithelial cell line A549. Although both CXCR1 and CXCR2 can mediate ERK activation (13, 14), the ability of MGSA/GROα and IL-8 to cause phosphorylation of ERK1/2 in human monocytes has not been investigated. The importance of this pathway in the autocrine production of IL-8 in mononuclear cells was demonstrated by the dose-dependent inhibition of IL-8 production by the MEK1/2 inhibitor PD-98509 (Fig. 5A). Because IL-8 but not MGSA/GROα was able to stimulate IL-8 production from MNCs, it was of interest to compare the abilities of each of these ligands with respect to phosphorylation of ERK1/2 in these cells. As in PMNs, phosphorylation of ERK in response to IL-8 was rapid and transient, peaking within 1 min. However, the phosphorylation of ERK1/2 after stimulation with equimolar concentrations of MGSA/GROα was barely detectable relative to IL-8 stimulation (Fig. 5B). These data are consistent with the efficacy of each ligand to induce IL-8 production and lend support to a role for ERK activation in autocrine IL-8 production.

DISCUSSION

Results obtained from this work establish IL-8 as an autocrine regulator of IL-8 production in monocytes. As such, IL-8 acts on one of its own receptors and stimulates the biosynthesis of IL-8 through a mechanism that involves de novo mRNA and protein synthesis. Monocytes and tissue macrophages are primary sources of inflammatory cytokines and chemokines. It is generally assumed that IL-8 released by cells at inflammatory sites diffuses to more distal regions where, at a subnanomolar concentration, it serves as a principal chemoattractant for inflammatory cells. In the lung, IL-8 is synthesized and released by epithelial

Fig. 5. Role of mitogen-activated protein kinase activation in autocrine IL-8 synthesis. A: effect of the mitogen-activated protein kinase kinase inhibitor PD-98059 on IL-8-induced IL-8 secretion as assessed by preincubating cells for 30 min with increasing amounts of the drug as indicated. Cells were subsequently stimulated with 10 μl of IL-8 beads for 2 h, and the supernatants were harvested for quantitation of IL-8. B, top: ability of MGSA and IL-8 to increase the levels of phosphorylated extracellular signal-regulated kinase (ERK) was determined by stimulating freshly isolated MNCs for different times followed by analysis of cell lysates by Western blotting with an anti-phospho-ERK (ERK-P) antibody. The prominent band represents ERK2, reflecting a size of 42 kDa. B, bottom: identical blot after reprobing with antibodies specific to the unphosphorylated form of ERK as a measure of protein loading.
cells (17) as well as by residential macrophages (29) and plays an important role in the recruitment of PMNs and MNCs (28). The discovery that MNCs produce subnanomolar concentrations of IL-8 after stimulation indicates the likelihood of autocrine signaling in vivo. Local production of IL-8 by the stimulated MNCs may facilitate the accumulation of this chemokine over time to a higher concentration than is required for activation of several phagocyte functions. In addition, the autocrine production of IL-8 by MNCs may amplify an inflammatory response by creating a possibly transient and self-sustaining source of this potent chemotactic factor, thus attracting additional leukocytes to the site of inflammation. It has been shown that MNCs may amplify their recruitment into inflammatory lesions by inducing self-production of monocyte chemotactic protein (8). Thus autocrine regulation of chemokines provides an additional mechanism for the recruitment of inflammatory cells. The in vivo production of IL-8 may be negatively regulated by cytokines such as IL-4 and IL-10. Future study will be necessary to determine the conditions under which IL-8 autocrine regulation occurs in vivo.

Our data suggest that autocrine IL-8 production is both cell specific and receptor specific. MNCs, which express less CXCR1 and CXCR2, respond better than PMNs in autocrine IL-8 production. The exact signaling mechanism that dictates this difference is not clear, but there are several possibilities. MNCs are a major source of cytokines and have a well-developed machinery for protein synthesis and secretion. In contrast, PMNs are terminally differentiated cells suited for special functions such as degranulation and superoxide generation. However, their protein synthesis capabilities are quite limited. Published studies indicate that PMNs utilize their remnant transcription and translation machinery to express a selected number of genes (12). Although PMN expression of IL-8 has been documented, the level of production is well below that of MNCs (3, 5). Another possibility is that PMNs and MNCs employ different signaling mechanisms downstream of the IL-8 receptors, which then lead to different cellular functions. In PMNs, IL-8 stimulation triggers the generation of superoxide anions and release of granule contents, whereas in MNCs, IL-8 stimulation causes production of IL-8 and possibly other cytokines. Consistent with this notion is the observation that IL-8 induces a more pronounced Ca\(^{2+}\) mobilization in PMNs, which is critical for such functions as degranulation.

Autocrine IL-8 production in MNCs preferentially utilizes CXCR1, which binds IL-8 but not MGSA/GRO\(\alpha\). The latter is a potent agonist for CXCR1 but not MGSA/GRO\(\alpha\). The CXCR1 ligand is tax gene product of human immunodeficiency virus-1. It is noted, however, that MGSA/GRO\(\alpha\) can serve as an autocrine regulator under some conditions such as in melanoma cell lines (24). We believe that preferential usage of CXCR1 in autocrine IL-8 production occurs in certain types of cells such as MNCs.

In summary, this study provides direct evidence that autocrine IL-8 production occurs in monocytes stimulated with IL-8 and that this cellular response is regulated at the cell and receptor levels. These new observations warrant additional studies of the signaling mechanisms activated by IL-8 and MGSA/GRO\(\alpha\). The finding may have profound applications in leukocytes and in other types of cells as IL-8 is more and more recognized as a regulator of cell proliferation in addition to its traditional role in chemotaxis.

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