Cytokine Regulation of Colony-Stimulating Factor Production in Cultured Human Synovial Fibroblasts: I. Induction of GM-CSF and G-CSF Production by Interleukin-1 and Tumor Necrosis Factor

By Tali Leizer, Jonathan Cebon, Judith E. Layton, and John A. Hamilton

The cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF), induce a dose-dependent production of both granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte CSF (G-CSF) in cultured human synovial cells, as measured by immunoassay. With IL-1, significant levels of both CSFs were first detected within 6 to 12 hours, with a maximum reached 24 to 48 hours after commencement of stimulation. A synergistic effect was detected between IL-1 and TNF in production of both CSFs in these cells. No evidence was obtained for the IL-1-induced effect to be mediated by induction of endogenous TNF nor for the TNF-induced stimulation to involve IL-1. IL-1-stimulated synovial cells were shown to secrete biologically active GM-CSF and G-CSF, which were specifically inhibited by their respective monoclonal antibodies. The

COLONY-STIMULATING factors (CSFs) are pivotal regulators of the survival, proliferation, differentiation and activation of hematopoietic cells. Human granulocyte-macrophage CSF (GM-CSF) is a glycoprotein with molecular weight ranging between 15,000 and 31,000 daltons, mediating the differentiation of progenitor cells into mature granulocytes and macrophages and the clonal proliferation of macrophage and neutrophil progenitors in vitro. Human granulocyte CSF (G-CSF) is an acidic 22,000-dalton glycoprotein originally identified by its stimulation of in vitro proliferation and differentiation of bone marrow progenitor cells into granulocytes. Besides sharing some similarities in their actions on hematopoietic cell lineages, GM-CSF and G-CSF can act on mature cells of these lineages. Both CSFs enhance antibody-dependent cytotoxicity, neutrophil oxidative metabolism, and microorganism phagocytosis by neutrophils. GM-CSF can also act on monocyte/macrophages, for example, to increase plasminogen activator activity, and to induce HLA-DR and HLA-DQ antigens.

GM-CSF has recently been reported in both synovial fluids and tissue culture supernatants from patients with rheumatoid disease and, on account of its ability to elevate monocyte 1a antigen, has been proposed to be important in the pathogenesis of the disease. However, the cellular source(s) of this activity in synovial tissues is unknown. Activated synovial fibroblast-like cells have been implicated in the invasive and erosive properties of the inflamed synovium of rheumatoid lesions. They have been shown in vitro to be activated by cytokines, such as interleukin-1 (IL-1), to produce a number of putative mediators of inflammation and tissue destruction, such as plasminogen activator, collagenase, IL-6, prostaglandin E2 (PGE2), and tumor necrosis factor α (TNFα). Many cytokines have been found in the rheumatoid synovium that could be acting on these cells. These include IL-1, IL-1β and TNFα, and TNFα has been reported to induce GM-CSF and G-CSF in certain other cell types, including those of nonhematopoietic origin. We have therefore determined whether synovial fibroblasts produce GM-CSF and G-CSF, and examined the effect of various cytokines on CSF production.

One major limitation of attempts to identify and quantify CSF activities in biological samples by bioassays is that these assays are often influenced by the presence of other molecules, including other cytokines. For this reason we decided to use sensitive immunoassays to measure GM-CSF and G-CSF in cultures of these cells. We report that IL-1α, IL-1β, TNFα, and TNFβ are all able to stimulate human synovocyte GM-CSF and G-CSF production in vitro and suggest that the stimulated fibroblast-like cell in the synovial tissue may be a source of these CSFs in rheumatoid arthritic lesions.

METHODS

Synovial cell cultures. Human synovial cell explant cultures were established from non-rheumatoid donors as previously described. Passaged cells were functionally and morphologically similar to the synovial fibroblast-like cells. In experiments, synoviocytes were plated overnight at 2 x 10⁵ cells/0.2 mL/well in 96-well plates (Linbro, Flow Laboratories, McLean, VA) in α-modified minimal essential medium (α-MEM) (Commonwealth Serum Laboratories, Parkville, Australia) supplemented with 10% heat-

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inactivated (at 56°C for 30 minutes) fetal bovine serum (FBS) (Flow). After washing with phosphate-buffered saline (PBS), pH 7.4, 20 µL cytokine samples were added to 200 µL α-MEM containing 1% FBS, which had been depleted of plasminogen by lysine-Sepharose chromatography to allow the separate measurement of plasminogen activator. 25 Synovial cells were usually incubated for a further 24 hours before supernatants were collected and assayed for GM-CSF and G-CSF.

Production of murine monoclonal antibodies to GM-CSF and G-CSF. Anti–GM-CSF antibodies, LMM102 and LMM111, together with anti–G-CSF antibody, LMM201, were produced according to the procedure outlined (Dempsey et al, manuscript submitted). Spleen cells from mice immunized with either recombinant human GM-CSF (rhGM-CSF) or rhG-CSF were fused with P3-NS1/1-Ag411 murine myeloma cells and the hybrid cells were propagated. Anti-CSF antibody-producing cells were cloned and injected intraperitoneally into mice to produce ascites. Antibodies were purified from ascites fluid by ammonium sulfate precipitation and Protein A-Sepharose affinity chromatography.

Sandwich enzyme-linked immunosorbent assay (ELISA) for GM-CSF. A sandwich ELISA assay was used to quantify GM-CSF production by human synovial cells. The assay was performed as described 29 in 96-well microtiter plates that were previously coated with purified monoclonal antibody, LMM111, blocked with bovine serum albumin (BSA), and stored dry at −20°C until use. Briefly, the assay involved incubation of 100 µL/well synovial cell supernatant in triplicate for 16 hours at 4°C, followed by washing in PBS containing 0.1% Tween 20. The plates were then incubated for 3 hours at 20°C, with rabbit anti-IGM-CSF serum (R4 1.12), diluted 1:1,500 in ELISA diluting buffer, and then with horseradish peroxidase-conjugated donkey anti-rabbit Ig (1:500, 1 hour; 20°C; Amersham, Amersham, England). After washing, peroxidase substrate ABTS solution (Sigma Chemical, St Louis, MO) was added to the plates and color development measured on a Titertek MCC/340 absorbance plate reader (Flow) at 405 nm, with a reference filter, 450 nm. Results are expressed as mean values ± SEM with a sensitivity limit of 0.02 ng/mL. In some experiments, an immunopurification kit, Insight-GM (MRL, Sydney, Australia), using the same antibodies and method, was used.

Immunopurification and bioassay of synovial cell GM-CSF. GM-CSF activity was determined by the proliferation assay of AML-193 human monocytic leukemia cells as published. 30 As this assay is susceptible to interference from multiple cytokines, it was first necessary to purify GM-CSF from synovial cell supernatants by immunoaffinity chromatography. 31,32 IL-1–stimulated synovial cell supernatant (8.25 mL) was loaded onto an anti–GM-CSF (LMM111) affinity column, with five passes of the supernatant over the column to ensure maximum binding. The column was washed with glycine-HCL buffer, pH 4.0, containing 0.1% Tween 20 (10 x column volume). Retained GM-CSF was then eluted with 2.0 mL glycine-HCL buffer, pH 2.0, containing 0.1% Tween 20. After the addition of 100 µL of BSA solution, the eluate was dialyzed against PBS for 16 hours at 4°C and sterile-filtered.

In the bioassay, AML-193 cells were seeded at 1 x 10⁴ cells/well in 96-well plates. Purified synovial cell GM-CSF was added in the presence of 1 µg/mL each of an anti–GM-CSF antibody (LMM102), anti–G-CSF antibody (LMM201), and a control isotype-matched antibody raised against a human mammary carcinoma cell line (C1Bl7) (P. Dempsey, Ludwig Institute, Melbourne, Australia) 30; the cells were incubated for 4 days, and then pulsed for 18 hours with 0.5 µCi/well ³H-thymidine (³H-TdR, Amersham). They were then harvested by automated cell harverster (Titertek, Flow) and isotope incorporation measured by liquid scintillation counting. Fifty units/mL GM-CSF activity represented 50% of maximal ³H-TdR incorporation.

G-CSF radioimmunoassay. G-CSF levels in synovial cell supernatants were measured in a solid-phase radioimmunoassay (RIA). 33 This was performed in strips of flat-bottomed wells (Immulon-2, Dynatech Laboratories, Alexandria, VA), previously coated with the IgG fraction of rabbit anti–G-CSF antibody, and blocked with BSA. Triplicate wells of samples and G-CSF standard were incubated for 5 hours before the addition of monoclonal anti–G-CSF (75A) for a further overnight incubation. Binding of antibody was detected by addition of ¹²⁵I–rabbit antirabbit IgG (NEN/Dupont, Boston, MA) for 2 hours. Measurement was by gamma-counting, with a sensitivity of 0.5 ng/mL G-CSF.

G-CSF bioassay. G-CSF activity was measured by a modification of the assay of the proliferation of the murine myeloid leukemia cell line, NFS-60. 37 Fifty-microliter assay samples (serially diluted 1:3) were added to 50 µL NFS-60 cells (1 x 10⁴ cells/mL) in DME media (Flow) containing 10% FBS (Flow) in 96-well plates. Assay samples were added in the presence of either 10 µg/mL anti–G-CSF monoclonal antibody (LMM201), or 1 µg/mL each of anti–GM-CSF antibody (LMM111), or control antibody (CIBr7). Cells were incubated for 24 hours at 37°C before the addition of 0.5 µCi/well ³H-TdR (specific activity, 5 Ci/mmol; Amersham), incubated for a further 6 hours, harvested with a Titertek automated cell harverster (Flow) onto filter paper and then assayed for ³H-TdR incorporation by beta-scintillation counting.

Cytokines and anticytokine antibodies. Purified recombinant human interleukin-1α (rhIL-1α) (specific activity of 5.7 x 10¹⁷ U/mg), human GM-CSF (rhGM-CSF) (1 U/mL ~ 10⁻¹² mol/L) and interleukin-12 (rhIL-12, 2.5 x 10⁸ U/mg) were the generous gifts of P. Lomedico, Hoffmann-La Roche, Nutley, NJ 34 and A. Shaw, Glaxo, Geneva, Switzerland, respectively. One unit of IL-1 activity was defined as the amount mediating half-maximal stimulation in the murine thymocyte Comitogenesis assay. 35 Recombinant TNFa (5 x 10⁷ U/mg) and TNFb (lymphotoxin, 10⁷ U/mg) were obtained from G.R. Adolf, Boehringer Ingelheim, Sydney, Australia. One unit of TNF activity was defined as the amount causing 50% cytotoxicity of actinomycin-D–treated L929 murine fibroblast monolayers. 36 Recombinant human CSFs were obtained from the following sources: GM-CSF (8 x 10⁷ colony-forming U/mg), J. Delamarter, Glaxo, Geneva, Switzerland; 37 G-CSF, A. Shaw, Thermo Scientific, Cambridge, MA; macrophage colony-stimulating factor (M-CSF/CSF-1), E. Hochuli, Hoffmann-La Roche, Basel, Switzerland; interleukin-2 (IL-2), Cetus; transforming growth factor α (TGFα) (0.55 mg epidermal growth factor (EGF) equivalents/mg TGFα), M. Winkler, Genentech, South San Francisco, CA; and EGF, E. Rich, Ludwig Institute, Melbourne, Australia. Purified native human platelet-derived growth factor (PDGF, an A-B heterodimer) was the gift of C-H. Heldin, Ludwig Institute, Uppsala, Sweden.

Goat antihuman IL-1α antiserum was obtained from R. Chizzonite, Hoffmann-La Roche, as above, with 1:100 dilution completely blocking 1,000 U/mL rhIL-1α in the D10.G4.1 helper T-cell proliferation assay. 38 Rabbit anti–IL-1β antiserum was kindly provided by A. Shaw, Glaxo, with a half-maximal LAF inhibitory titer of 1:200. Monoclonal TNFa antibody, with a neutralization titer of 6,000 U TNFa/mg antibody, was obtained from Genentech.

All cytokines and antisera were negative (<10 pg/mL) for endotoxin contamination in the Limulus lysate assay (Commonwealth Serum Laboratories).

Statistical methods. With the exception of the synergy between
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RESULTS

Cytokine induction of synoviocyte GM-CSF and G-CSF production. The cytokines, IL-1 and TNFα, stimulated the production of both GM- and G-CSF in cultured human synovial cells. IL-1α induced both CSFs in a dose-dependent manner (Fig 1). A similar dose titration was seen with IL-1β (not shown). TNFα and TNFβ (lymphotoxin) (≥10⁻¹⁰ mol/L and ≥10⁻⁶ mol/L, respectively) were also found to be active (Figs 2A and B); for GM-CSF for 10 of 16, and for G-CSF with 6 of 12 cell lines, TNFα did not induce levels of CSF levels that were within the detectable limits of the immunoassay. When CSF production in four responsive synoviocyte preparations was compared, IL-1 was found to be about 10 times more potent than TNFα and 1,000 times more so than TNFβ on a molar basis. Also, the maximal CSF secretion seen with the TNFs was always significantly less than that with the IL-1s. Neither CSF was detectable in synovial cell lysates (not shown).

The possibility that any part of the CSF-stimulating capacity of IL-1 or TNFα might be a consequence of cross-induction of the production of the other cytokine by the target synovial cells was tested (Table 1). Addition of a combination of anti-IL-1α and anti-IL-1β antisera (sufficient to neutralize 100 U/mL of each IL-1) to an optimal dose of TNFα failed to alter production of either CSF, suggesting that TNFα (or TNFβ) does not stimulate synoviocyte GM-CSF and G-CSF levels through an autocrine IL-1-dependent mechanism. This is supported by our failure to detect IL-1 activity in TNF-stimulated synovial cell supernatants, as measured in the murine thymocyte mitogenesis assay (results not shown). Likewise, antibodies to TNFα did not affect IL-1-stimulated GM-CSF and G-CSF production. TNF activity was not detected in IL-1-stimulated supernatants, as measured in the L929 cytotoxicity assay (not shown), indicating that any IL-1-mediated induction of synoviocyte TNFα would not account for the IL-1 effect.

Synergy between IL-1 and TNF in synoviocyte CSF production. To further analyze the mechanism of cytokine-
were also cultured with TNFα (10⁻¹ mol/L) that had been preincubated with preimmune serum, anti-TNFα, or anti-IL-la (1:100). Cells were incubated for 24 hours before supernatants were collected and assayed for GM-CSF and G-CSF by immunoassay with an anti-GM-CSF antibody (LMM102), or an anti-G-CSF antibody (LMM201). The biological activity of GM-CSF (mean ± SEM, n = 3) was recovered from the affinity column in the presence of CIBr7 control antibody was 16.1 ± 2.5 × 10¹⁰ U/mL. In the presence of anti-GM-CSF antibody, LMM102, 1.7 ± 1.3 × 10¹¹ U/mL (P < .005) of GM-CSF activity was present. In contrast, anti-G-CSF antibody, LMM201, failed to inhibit ³H-TdR incorporation with 19.5 ± 2.3 U/mL detected (not significant). These results confirm that synovial cell supernatant GM-CSF was both bioactive and specifically inhibited by an anti-GM-CSF monoclonal antibody.

Likewise, a granulocyte colony-stimulating activity, detected in IL-1α-stimulated synovial cell supernatants, was inhibited (~90%) by anti-G-CSF (LMM201) antibody (Fig

### Table 1. The Effect of Anticytokine Antisera on IL-1 and TNFα-Stimulated CSF Production

<table>
<thead>
<tr>
<th>Condition</th>
<th>GM-CSF (ng/mL)</th>
<th>G-CSF (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02 ± 0.01</td>
<td>0.1 ± 0</td>
</tr>
<tr>
<td>Anti-IL-1α + anti-IL-1β</td>
<td>0.03 ± 0.01</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Anti-TNFα</td>
<td>0.02 ± 0.02</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>IL-1α (100 U/mL)</td>
<td>0.98 ± 0.06</td>
<td>17.9 ± 0.3</td>
</tr>
<tr>
<td>IL-1α + anti-IL-1α</td>
<td>0.05 ± 0.02</td>
<td>1.4 ± 0.04</td>
</tr>
<tr>
<td>IL-1α + anti-TNFα</td>
<td>1.01 ± 0.06†</td>
<td>17.3 ± 0.4†</td>
</tr>
<tr>
<td>TNFa (10⁻¹ mol/L)</td>
<td>0.45 ± 0.12</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>TNFa + anti-TNFα</td>
<td>0.02 ± 0.02†</td>
<td>0.2 ± 0.0*</td>
</tr>
<tr>
<td>TNFa + anti-IL-1α + anti-IL-1β</td>
<td>0.43 ± 0.02†</td>
<td>6.6 ± 0.1†</td>
</tr>
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</table>

Synoviocytes (from donor LH, passage 3) were cultured with IL-1α (100 U/mL) that had been preincubated (1 hour, 37°C) with preimmune serum (1:10), anti-IL-la (1:1,000), or anti-TNFα (1:100). The cells were also cultured with TNFa (10⁻² mol/L) that had been preincubated with preimmune serum, anti-TNFα, or a combination of anti-IL-1α and anti-IL-1β (1:100). Cells were incubated for 24 hours before supernatants were collected and assayed for GM-CSF and G-CSF by immunoassay (mean ± SEM, n = 3).

*P < .001 compared with cytokine control.
†P > .05 compared with cytokine control.
‡P < .015 compared with cytokine control.

For GM-CSF production in 12 experiments (see Methods), there was a significant main effect for IL-1 alone of 0.52 ± 0.09 ng/mL (P < .001), for TNFa (10⁻² mol/L) alone, 0.12 ± 0.06 (not significant), and for IL-1 + TNFa of 1.08 ± 0.17 ng/mL. The calculated interaction effect (IL-1 + TNFa) − (IL-1) − (TNFa) was 0.44 ± 0.20 ng/mL (P < .03). Likewise, for G-CSF production, the main effect of IL-1 alone was 9.34 ± 1.63 ng/mL (P < .001), of TNFa alone 0.84 ± 0.02, and of IL-1 + TNFa 20.17 ± 3.65 ng/mL with an interaction effect of 10.00 ± 4.01 ng/mL (P < .02).

**Kinetics of monokine-stimulated CSF production.** The time-course of cytokine-stimulated synovial cell CSF production was investigated. A similar kinetics profile was seen for the secretion of both GM-CSF (Fig 4A) and G-CSF (Fig 4B) with IL-1α, TNFa, and IL-1α + TNFa. With IL-1α, significant synoviocyte release of either CSF was first detected within 6 to 12 hours of stimulus, reaching a maximum at 24 to 48 hours. The synergy between IL-1 and TNF also became apparent within 6 hours of addition, with a maximal response detected at 24 to 48 hours.

**Biological activity of synoviocyte CSFs.** To determine whether the synovial cell CSFs detected by ELISA and RIA were biologically active, we titrated supernatants in respective biological assays in the presence of anti-CSF antibodies. GM-CSF bioactivity was measured in the AML-193 proliferation assay (see Methods). We extracted GM-CSF from synovial cell supernatants using immunoadfinity chromatography with a specific monoclonal GM-CSF antibody, LMM111. The column eluate was added to the AML-193 assay in the presence of a control antibody (CIBr7), a different anti-GM-CSF antibody (LMM102), or an anti-G-CSF antibody (LMM201). The biological activity of GM-CSF (mean ± SEM, n = 4) recovered from the affinity column in the presence of CIBr7 control antibody was 16.1 ± 2.4 × 10¹¹ U/mL. In the presence of anti-GM-CSF antibody, LMM102, 1.7 ± 1.3 × 10¹¹ U/mL (P < .005) of GM-CSF activity was present. In contrast, anti-G-CSF antibody, LMM201, failed to inhibit ³H-TdR incorporation with 19.5 ± 2.3 U/mL detected (not significant). These results confirm that synovial cell supernatant GM-CSF was both bioactive and specifically inhibited by an anti-GM-CSF monoclonal antibody.

![Fig 3. Synergy between IL-1 and TNFα in synovial cell GM-CSF production. Synovial cells (from donor LH, passage 6) were incubated with TNFa (10⁻⁹ mol/L) supplemented with 1 to 1,000 U/mL IL-1α (A), or cultured with IL-1 (100 U/mL) supplemented with 10⁻⁹ to 10⁻¹¹ mol/L TNFa (B), and supernatant GM-CSF concentration measured (±SEM, n = 3).](image-url)
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5), showing that stimulated synovial cells secrete biologically active G-CSF.

Effect of transcriptional and translational inhibitors on IL-1-stimulated CSF production. The mechanism of synovial cell CSF production was investigated to determine whether cytokine-stimulated supernatant GM-CSF and G-CSF levels represented newly synthesized protein or protein released from preformed cellular stores. Nontoxic doses of both the RNA synthesis inhibitor, actinomycin D, and the protein synthesis inhibitor, cycloheximide, abolished IL-1-mediated GM-CSF and G-CSF production when measured at 8 hours (Table 2), suggesting that IL-1 stimulated de novo GM-CSF and G-CSF synthesis. These findings are consistent with the observation that no CSFs were detected in the lysates of untreated cells at the start of the incubation. Indeed, no detectable levels of GM-CSF or G-CSF were measured in synovial cell lysates after any treatment mentioned in this article, suggesting that both CSFs are inducible with no significant intracellular stores.

The effect of other cytokines on IL-1 or TNFα-mediated CSF production. The following cytokines failed to stimulate GM-CSF and G-CSF secretion by cultured synovial cells, either alone or in combination with IL-1 or TNF after 24 hours' incubation: M-CSF (CSF-1) (10 to 1,000 U/mL), IL-3 (5 to 500 U/mL), IFNγ (50 to 5,000 U/mL), IL-2 (50 to 5,000 U/mL), PDGF (10⁻¹⁰ to 10⁻⁹ mol/L), EGF, and TGFα (5 to 500 ng/mL). Neither GM-CSF (10 to 1,000 U/mL) nor G-CSF (0.1 to 1 μg/mL) induced the production of the other CSF, suggesting that the CSFs do not cross-induce the production of each other by synovial cells.

DISCUSSION

In this study, we have shown that IL-1 and TNF stimulate human synovial fibroblast-like cells to produce GM-CSF and G-CSF. Immunoassays were used to quantitate the level of the CSFs because there are limitations to the bioassays used to identify and quantitate them. For example, the classical bone marrow colony-forming assay for GM-CSF is susceptible to variability between sources and within cultures, takes up to 14 days, and is influenced by other cytokines, such as IL-1 and IL-6.14 The alternative bioassay for GM-CSF, involving the proliferation of acute myelogenous leukemia 193 cells, is also subject to interference from multiple cytokines, including IL-1 and IL-627 (unpublished data). Because we add IL-1 to some of our cultures and because IL-6 is produced by our synovial cells (unpublished data),4 the immunoassays were routinely used. The sensitive imm-

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Table 2. Transcriptional and Translational Inhibitors and IL-1-Induced CSF Production

<table>
<thead>
<tr>
<th>Condition</th>
<th>GM-CSF (ng/mL)</th>
<th>G-CSF (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.01 ± 0</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>IL-1 (100 U/mL)</td>
<td>1.30 ± 0.10</td>
<td>11.2 ± 5.5</td>
</tr>
<tr>
<td>IL-1 + actinomycin D</td>
<td>0.01 ± 0*</td>
<td>1.0 ± 0.1*</td>
</tr>
<tr>
<td>IL-1 + cycloheximide</td>
<td>0.01 ± 0*</td>
<td>0.7 ± 0.1*</td>
</tr>
</tbody>
</table>

Synovial cells (from donor LH, passage 4) were incubated with IL-1α (100 U/mL) supplemented with media, actinomycin D (1 μg/mL), or cycloheximide (1 μg/mL) for 8 hours before supernatants were collected and assayed for GM-CSF and G-CSF by immunoassay (mean ± SEM, n = 3). *P < .0001, compared with IL-1 treated control.
no assays provide advantages of low picomolar sensitivity, rapid quantitation, large sample number capacity, and lack of interference from other cytokines present in the synovial cell cultures.

IL-1 has been reported to stimulate the production of a GM-colony stimulating activity in cultured human lung fibroblasts and production of both GM-CSF and G-CSF in dermal and bone marrow fibroblasts. Likewise, TNFα was shown to elevate both GM-CSF and G-CSF mRNA and secreted protein levels in human lung fibroblasts. Our data indicate that IL-1α, IL-1β, TNFα, and TNFβ are all active in increasing the levels of both GM-CSF and G-CSF with evidence of synergy between IL-1 and TNF. The latter is contrary to the findings of Seelentag et al., who described an additive effect for TNF stimulation of fibroblast GM-CSF and G-CSF mRNA. The synergistic effect between IL-1 and TNF would suggest the possibility of posttranscriptional amplification processes with the presence of only small amounts of both cytokines being able to induce CSF production.

In order to determine whether the immunoreactive GM-CSF and G-CSF measured in stimulated synoviocyte supernatants by ELISA or RIA were biologically active, respective bioassays were used. In the case of GM-CSF, it was necessary to include a preliminary immunopurification step to isolate a GM-colony stimulating activity (CSA) which was then found to be specifically inhibited by a neutralizing anti-GM-CSF antibody. Similarly, a G-CSF which was inhibited by an anti-G-CSF antibody was identified.

Several cytokines, which have been detected in rheumatoid lesions, were tested for their effects on CSF production, but the IL-1s and TNFs were the only cytokines that were active; in other words, there is some specificity in the control of synoviocyte CSF synthesis by cytokines. PDGF, for example, which is mitogenic for the synoviocytes, cannot activate them for CSF production. Synovial cells therefore can be included in the list of cell types, including other cells of mesenchymal origin, that can respond to IL-1s and TNFα by increasing GM-CSF and G-CSF production.

The synthesis of other synovial fibroblast products is enhanced by the IL-1s and TNFs, including plasminogen activator, collagenase, prostanoids, hyaluronic acid, and IL-6. We suggest that synovial fibroblast-like cells, under the influence of IL-1s and TNFα, either alone or in combination, may contribute to the levels of GM-CSF and other uncharacterized CSF(s) present in rheumatoid synovial fluids. The GM-CSF produced by the activated synoviocytes could in turn activate monocytes in such lesions, for example, by stimulating plasminogen activator activity, class II major histocompatibility complex antigen expression, and monokine release. In vitro, GM-CSF enhances monocyte adherence to the endothelium, which could explain the transient and rapid monocytopenia found after intravenous injection of GM-CSF into patients. Such enhanced monocyte-endothelial adhesion by locally produced GM-CSF may also be involved in the observed close apposition of monocytes to the blood vessel wall and also in the altered transendothelial migration and intrasynovial accumulation of monocytes into rheumatoid joints. Recently, GM-CSF administered to a patient with Felty's syndrome was associated with a flare-up of rheumatoid arthritis and release of cytokines. Furthermore, both GM-CSF and G-CSF could activate neutrophils in rheumatoid synovial fluids, which may also contribute to the pathogenesis of the disease.

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