Cytokines in Chronic Inflammatory Arthritis

II. Granulocyte-Macrophage Colony-stimulating Factor in Rheumatoid Synovial Effusions

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Abstract

A liquid culture technique was used to study 23 synovial fluids (SF) (21 from inflammatory joint diseases and 2 noninflammatory SF) and supernatants of two cultured rheumatoid arthritis (RA) synovial tissues for colony-stimulating factor (CSF). The proliferative responses of human peripheral blood macrophage-depleted non-T cells treated with synovial fluids, supernatants of synovial tissue explants, and recombinant granulocyte-macrophage (rGM)-CSF were compared. Aggregates of cells that formed in long-term cultures (15 d) were similar for each applied agent and consisted of macrophages, eosinophils, and large blasts. Tritiated thymidine incorporation was proportional to the concentration of rGM-CSF and was accompanied by an increase in number and size of cellular aggregates formed in the cultures. CSF activity was observed in inflammatory SF, with tritiated thymidine uptake of 3,501±1,140 cpm in the presence of RA samples (n = 15) compared to 1,985±628 for non-RA inflammatory SF (n = 7) ($P < 0.05$) and 583±525 for medium (n = 6) ($P < 0.01$). The proliferative response to RA SF was often more apparent when the samples were diluted, because at higher concentrations the RA SF was inhibitory. Two RA SF were fractionated by Sephadex G100 column chromatography; low levels of CSF activity were detected in fractions corresponding to M_r of 70–100 kD, but the major CSF activity was found in the 20–24-kD fractions. A polyclonal rabbit anti-GM-CSF antibody eliminated the stimulating activity from both rGM-CSF and RA SF. Finally, a specific RIA identified significant levels of GM-CSF (40–140 U/ml) in the culture supernatants of 3 additional RA synovial tissues. These data document the local production of GM-CSF in rheumatoid synovitis and are the first description of this cytokine at a site of disease activity.

Introduction

Both the humoral and cellular arms of the immune response are involved in the pathogenesis of rheumatoid arthritis (RA).[1] While polymorphonuclear leukocytes and their products predominate in rheumatoid synovial fluid, mononuclear inflammatory cells participate in articular tissue destruction and inflammation (1). Our recent investigations have focused on the soluble mediators (i.e., cytokines) that result from these cellular interactions (2). A surprising finding from these studies is that only very small quantities of γ-interferon are found in the synovial fluid or membrane (3). Another important T cell product, namely IL-2, is undetectable in these tissues (4), whereas macrophage colony-stimulating factor (CSF-1) and a mast cell growth factor are demonstrated (5). Granulocyte-macrophage CSF (GM-CSF) is another hematopoietic growth factor that has additional interesting properties including potent stimulating effects on mature granulocytes and macrophages and enhancement of antigen presentation by accessory cells (6). We report here that chronic inflammatory joint effusions and supernatants of cultured RA synovial tissues contain a CSF activity which is similar to that seen with recombinant human (rh) GM-CSF. The activity resides in a fraction with the identical molecular weight of GM-CSF and is removed by antibody to GM-CSF. This is the first report of significant levels of GM-CSF at an involved site in an inflammatory disease. The implications of this finding for the perpetuation of chronic inflammatory synovitis are discussed.

Methods

Reagents. rhGM-CSF was provided by Amgen Biologicals (Thousand Oaks, CA). Rabbit anti-human GM-CSF was a kind gift from Dr. David Golde, (Los Angeles, CA). SC2 (monoclonal anti-DR antibody) was a gift from Dr. Robert Fox (Scripps Clinic and Research Foundation, La Jolla, CA). Monoclonal anti-macrophage antibody (63D3) was purchased from Bethesda Research Laboratories (Gaithersburg, MD) and OKT3 was obtained from Ortho Diagnostics (Raritan, NJ). Rabbit complement was purchased from Cedar Lane (Hornby, Ontario, Canada). Culture medium was RPMI 1640 (CORE, La Jolla, CA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM) and 10% heat-inactivated human AB serum (Pel-Freez, Rogers, AK). 24-well culture plates and 96-well flat bottom plates from Flow Laboratories (McClean, VA) were used. Wright-Giemsa stain pack was purchased from VWR Scientific, Inc. (San Francisco, CA). Ficoll-Hypaque was from Pharmacia Fine Chemicals (Piscataway, NJ) and lymphopaque from Accurate Chemical & Scientific Corp. (Westbury, NY). Heparin was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ).

Clinical protocol. Synovial fluids (SFs) from patients with definite or classical RA (13 cases), psoriatic arthritis (2 cases), Reiter's syndrome (3 cases), ankylosing spondylitis (2 cases), and osteoarthritis (2 cases) were studied. All patients met the standard criteria for diagnosis and all joint aspirations were clinically indicated and collected according to procedures approved by the University of California, San Diego Human Subjects Committee. Heparinized SFs were centrifuged 20 min at 2,000 rpm. The cell-free samples were aliquoted, stored at −70°C until used, and used only once. Plasma samples from seven normal individuals were pooled and served as controls. Synovial tissue (ST) was obtained at the time of joint replacement surgery from patients with RA. Single-cell suspensions of synovial cells were prepared

Received for publication 8 February 1988 and in revised form 8 September 1988.

1. *Abbreviations used in this paper:* GM-CSF, granulocyte-macrophage colony-stimulating factor; NT, non-T cell; RA, rheumatoid arthritis; SF, synovial fluid; ST, synovial tissue.

J. Clin. Invest.

0021-9738/89/03/0876/07 $2.00
Volume 83, March 1989, 876–882

as previously described (3). The cells were incubated in RPMI 1640 supplemented with 10% endotoxin-free fetal calf serum (J. R. Scientific, Woodland, CA) and cultured for 3 d at 37°C and 5% CO_2. Supernatants were harvested, aliquoted, and frozen at −70°C until assayed.

Cell preparation. Peripheral blood was collected into sterile heparinized syringes and mononuclear cells were separated by centrifugation on Ficoll-Hypaque density gradients. Interface cells were washed twice in RPMI 1640 with 10% heat-inactivated human AB serum. Nonadherent T cells were isolated by passing 1×10^8 cells through a 0.6 g nylon wool column. The effluent T cells obtained by this method were then treated with an anti-Ia monoclonal antibody and fresh rabbit complement for 60 min at 37°C. The non-T cell (NT) population was obtained by first washing the column with 30 ml of serum-free RPMI 1640 to remove any residual T cells and then incubating the column at 4°C for 30 min. The NT were collected by rinsing the nylon wool column with ice-cold RPMI 1640 several times. Macrophages were subsequently removed from the NT population by two consecutive adherence steps to plastic petri dishes for 1 h at 37°C. After these treatments the T cells were > 95% $OKT3^+$ and < 2% SC2 (HLA-DR)-positive as determined by fluorescence microscopy. The NT population contained about 5% $OKT3^+$ cells, 2–3% macrophages ($63D3^+$ cells), 12% $B1^+$ cells (B cells) (Coulter Immunology, Hialeah, FL), and 25% Leu 11^+ cells (NK cells) (Becton-Dickinson, Mountain View, CA).

Liquid CSF assay. Purified T or NT cells (1.5×10^6/1.5 ml) were cultured separately or together in medium containing 10% human AB serum in 24-well plates for 15–20 d at 37°C in a fully humidified atmosphere with 5% CO_2. Recombinant hGM-CSF, SF, or plasma was added at the initiation of cultures. Half the medium in the cultures was replaced every 7 d with 0.75 ml of medium alone, or medium with GM-CSF or SF. Cultures were examined every 3–5 d and direct cell counts were performed on replicate wells using an inverted-phase microscope at ×200 magnification. Under these conditions viable cells had a sharp, clearly defined cell border and were highly refractile, whereas dead cells were dull, had no distinct edge, and had sparse cytoplasm. Small (10–50 cells) and large aggregates (> 50 cells) of cells were also counted in the wells on day 10 and 15. In some experiments rabbit anti-human GM-CSF antibody (1:50) was added at the initiation of the culture to block the activity of GM-CSF and SF.

Morphology of cells growing in the CSF assay. Cytospin preparation of cells grown with rGM-CSF or SF were prepared from pooled contents of three to four wells. These were stained with Wright-Giemsa, or fixed and stained for nonspecific and chloroacetate esterase as previously described (7).

Enumeration of cells and [^3H]TdR incorporation. Cells were removed from culture on days 10 and 15 and sedimented on lymphopaque to remove dead cells. The interface cells were washed three times with medium and concentrated by centrifugation for 10 min at 500 rpm. Viable cells were counted with a hemocytometer and trypan blue dye. Slides were made by cytospin and stained with Wright-Giemsa. The characteristics of the colony-forming cells was assessed by analyzing 500 cells.

[^{3}H]TdR incorporation by macrophage-depleted NT cells was determined by taking 0.2 ml of each cell suspension from culture and adding it to a 96-well plate on day 9 and 14. Cells were pulsed with [^{3}H]TdR (1 μCi/well) for 12 h, harvested onto glass fiber filters, and counted in a scintillation counter. All determinations were performed in triplicate.

Clonal proliferation of normal human marrow granulocyte-macrophage stem cells. Nonadherent bone marrow mononuclear cells 1–2 $\times 10^5$/ml from normal donors were plated in triplicate 1 ml cultures with 5% SF or synovial tissue supernatants and incubated in 35-mm tissue culture dishes in 7.5% CO_2 at 37°C for 14 d as described (7). Dilutions of a single lot of placenta conditioned medium prepared according to the procedure of Shlunk and Schleyer (8) were included as a positive control. Colonies (> 40 cells) and clusters (10–40 cells) were scored on the 7th and 14th days of incubation.

Fractionation of SF. 2–5 ml of SF from two patients with RA was concentrated fivefold, dialyzed against PBS, and fractionated on a Sephadex G100 column (75 × 1.5 cm). The column was initially equilibrated with PBS (pH 7.4); after the sample was applied, the column was washed with an additional 150 ml of PBS. A flow rate of 10 ml/h was maintained and fractions of 3 ml each were collected. The molecular weights of the various fractions were determined by comparison with standards (ferritin 440,000; bovine serum albumin 67,000; ovalbumin 43,000; ribonuclease A 13,700). Fractions were collected, sterilized by filtration, and tested in the liquid CSF assay. Aggregates of cells were counted on day 15.

GM-CSF radioimmunoassay. GM-CSF was quantified using a sandwich radioimmunoassay. The assay relies on two non-cross-reacting murine monoclonal antibodies raised by immunization with recombinant human GM-CSF (9). The antibodies, both IgG1 subclass, are specific for human GM-CSF and were purified by Staph A affinity chromatography. The first antibody was plated in microtiter wells, and then nonspecific protein adherence was blocked by the addition of 2% albumin. Culture supernatants were added to 10% final volume, allowed to adsorb, and after washing, I-125 labeled second antibody (iodobead method) was added. After washing the excess labeled antibody, individual wells were counted. The results were compared to standards containing rhGM-CSF titered for activity in human marrow colony-forming assays (1×10^8 U/mg). The assay does not recognize M-CSF, G-CSF, or human IL-3. The limit of sensitivity is 10 U/ml.

Statistics. Statistical analysis was performed by Student's *t* test. Data are presented as mean±standard deviation.

Results

While studying the effect of human SF and various growth factors on the autologous mixed leukocyte reaction it was observed that, although most of the original cells had died by day 7, the cultures contained small collections of large, round, refractile cells. These cells increased in number over the next 2 wk and appeared to expand from a central focus to form small (10–50 contiguous cells) or large aggregates (> 50 contiguous cells). To determine the origin of the cells responsible for aggregate formation, isolated populations of peripheral blood T and macrophage-depleted NT cells were cultured with SF or GM-CSF. Fig. 1 shows that if NT or T + NT were stimulated with SF or GM-CSF the number of new cells increased over

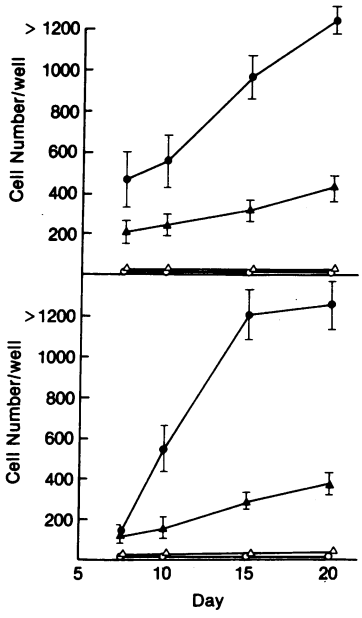

Figure 1. 2×10^5/well purified T and macrophage-depleted NT cells from normal donors were cultured in 96-well plates with medium and with 80 U/ml rhGM-CSF (*upper*) or 5% RA SF (*lower*). Viable cells were counted with an inverted-phase microscope on days 7, 10, 15, and 20. Results are the mean of four experiments using cells of separate donors. (● --- ●) NT + GM-CSF or SF; (▲ --- ▲) T + NT + GM-CSF or SF; (○ --- ○) NT alone; (△ --- △) T + GM-CSF.

the course of 20 d. This phenomenon was not observed with T cells alone, either in medium or when stimulated with SF or GM-CSF. There was even a suggestion (Fig. 1) that T cells suppressed aggregate formation. Lesser numbers of large aggregates also developed when whole PBM were used. Pretreatment of the NT cells with mitomycin C prevented the subsequent proliferation. These studies demonstrate that the progenitors of cell aggregates exist mainly in the NT population of peripheral blood.

Other variables in the liquid CSF assay were analyzed. The influence of the amount of GM-CSF on the number of cells as compared to cell division (measured by [^{3}H]thymidine [TdR] incorporation) was tested in two normal individuals and two RA patients. The results were similar in both groups. There was a direct relationship between cell numbers and increasing concentrations of GM-CSF (see Table I for a representative experiment). This was paralleled by an increase in [^{3}H]TdR incorporation (Fig. 2). The proliferative response was a function of the number of NT cells in the culture when there was a fixed amount (40 U/ml) of GM-CSF (Fig. 3). Therefore, unless otherwise specified, the liquid CSF assay was performed with 1 $\times$ 10^6/ml macrophage-depleted NT cells cultured with medium alone, medium plus 80 U/ml of GM-CSF, or medium plus 5% SF.

Morphology of the cells in cultures with GM-CSF and SF. Synovial fluid and GM-CSF stimulated cultures contained predominantly three types of cells (Table II). Cytospin preparations showed primitive blastlike cells, some with sparse granules (Fig. 4 *A*), macrophages (Fig. 4 *B*), and cells containing coarse, basophilic granules consistent with eosinophil precursors (Fig. 4 *C*). The latter cell type could potentially include basophil progenitors as previously described (10). Macrophages showed positive staining with nonspecific esterase, but only extremely rare chloroacetate esterase-positive cells were observed on the slides examined. The morphology and staining pattern of these cells were similar to those observed in a previous study of CSF-stimulated, human bone marrow in liquid culture (7).

Analysis of SFs. Twenty-four samples, including two supernatants of RA (ST) explants, were examined in the liquid CSF assay (Fig. 5). The most activity was found in the 13 SF and 2 ST derived from RA patients ([^{3}H]TdR = 3,501±1,140 cpm), compared to 583±525 cpm for medium control (n = 6; $P < 0.01$). Although there was some overlap, the results with

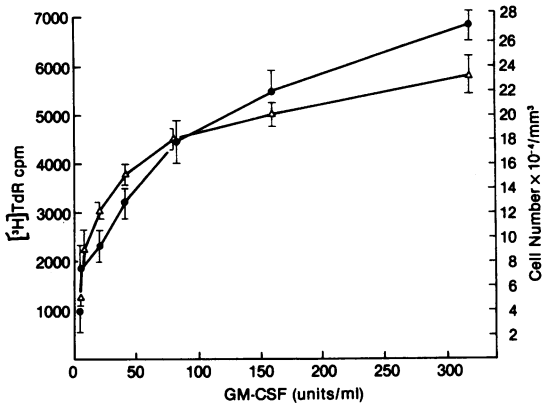

Figure 2. A comparison of cell numbers (△ —— △) and [^{3}H]Tdr incorporation (● --- ●) by macrophage-depleted NT cells cultured with medium plus various concentrations of GM-CSF. The cells were counted in a hemocytometer with trypan blue dye on day 15. Data represent the mean of four experiments with separate normal donors.

RA SF were significantly higher than those obtained with SF from other chronic inflammatory forms of synovitis (1,985±628 cpm, n = 7) ($P < 0.05$). A small amount of activity was found in two fluids from osteoarthritis patients ([^{3}H]Tdr = 1,677 cpm), but this was similar to the stimulation observed with pooled plasma from seven normal individuals ([^{3}H]Tdr = 1,175 cpm, $P > 0.1$) (data not shown). The dose responses of six RA SF were examined in the CSF assay and two distinct patterns were observed. In the first (Fig. 6 *A*) cell numbers and thymidine incorporation increased as the concentration of synovial fluid increased. This pattern was seen with only one fluid. In the remaining five there was a dose response at low SF concentrations followed by inhibition as the amount of SF increased (Fig. 6 *B*). No studies have been performed yet to determine whether the phenomenon observed in Fig. 6 *B* represents toxic factors or specific inhibitors in synovial fluid.

Semi-solid phase CFU-GM assay. Two ST supernatants were tested in a conventional human bone marrow CFU-GM assay and both were positive (see Table III for a representative experiment). None of five SF assayed supported colony formation, although clusters were abundant (mean of 202±46 clusters/10^5 cells for the five SF compared to 0 clusters in medium controls).

Table I. Effect of GM-CSF on Non-T Cell Growth

GM-CSF	Day 10	Day 15
U/ml	*cells/mm^3*	*cells/mm^3*
0	0	0
2.5	4×10^4	8×10^4
5.0	6×10^4	10×10^4
20.0	9×10^4	12×10^4
40.0	11×10^4	15×10^4
80.0	15×10^4	19×10^4

1.5×10^6 in 1.5 ml of medium macrophage-depleted NT cells from a normal donor cultured with different concentrations of GM-CSF in 24-well plates. 0.1 ml of cell suspension was removed and counted in a hemocytometer with trypan blue dye.

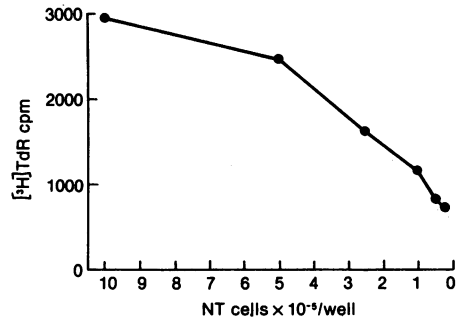

Figure 3. [^{3}H]Tdr incorporation observed on day 15 when varying numbers of normal macrophage depleted NT cells were cultured with medium containing GM-CSF 40 U/ml.

*Table II. Cell Growth with RA Synovial Fluids and Colony-stimulating Factor**

Donor	Stimulus	Cell types
1	GM-CSF	Macrophage, eosinophil
	SF1	Macrophage, blast
	SF2	Macrophage, granulocyte
	SF3	Macrophage, blast
	SF4	Macrophage, blast
	SF5	Eosinophil, macrophage
2	GM-CSF	Eosinophil
	SF3	Eosinophil, lymphocyte
	SF6	Macrophage, eosinophil, granulocyte
3	GM-CSF	Macrophage, eosinophil, granulocyte
	SF7	Eosinophil, macrophage, granulocyte
	SF8	Macrophage, eosinophil
	SF9	Macrophage, eosinophil

* NT cells from a normal donor were cultivated with either GM-CSF or RA SF for 2 wk.

GM-CSF radioimmunoassay. To confirm the production of GM-CSF by rheumatoid synovium, supernatants from three additional ST were assayed using a sensitive and specific RIA (see Table IV). One of the three ST was separated into adherent (> 70% nonspecific esterase-positive/< 10% $CD3^+$) and nonadherent populations (< 1% nonspecific esterase-positive/> 50% $CD3^+$), and the supernatants were harvested after 3 d. Although both populations of cells made GM-CSF, the adherent cells were more efficient (nonadherent cultures contain about fivefold more cells than adherent cultures). Nine additional RA SF were assayed in the RIA, and immunoreactive GM-CSF was below the level of detection.

Identification of the SF factor responsible for aggregate formation. Because our biological assays were 10-fold more sensitive than the RIA, we felt that the SF activity could still be due to GM-CSF. To study this possibility, the CSF was characterized by column chromatography and by antibody neutralization studies. Two RA SF were fractionated on a Sephadex G100 column. The majority of the protein eluted in the first 20 fractions (Fig. 7). In both cases a small number of aggregates were observed in these high molecular weight fractions, but the majority of the activity resided in fractions with an apparent molecular mass of 20–24 kD, which is similar to that of GM-CSF.

In a second set of experiments, polyclonal rabbit anti-human GM-CSF antibody was tested for its ability to remove the CSF activity from three RA synovial fluids (Table V). A 1:50 dilution of the rabbit antibody introduced at the initiation of culture eliminated the CSF activity of both recombinant GM-CSF and SF. Control rabbit serum had no effect and the antibody was not toxic when added to proliferating cells in culture (data not shown). Furthermore, in the human bone marrow CSF assay, polyclonal rabbit antibody to GM-CSF completely neutralized the CSF activity in RA SF (data not shown). Therefore, specific antibody to GM-CSF neutralized the CSF activity in RA SF demonstrated in both of our biological assays.

Discussion

The response of macrophage depleted peripheral blood non-T cells to recombinant GM-CSF or SF was qualitatively similar to that of human bone marrow or chronic myeloid leukemia cells to crude or purified CSF (7, 11). In each assay, [^{3}H]TdR incorporation was cell number- and CSF concentration-de-

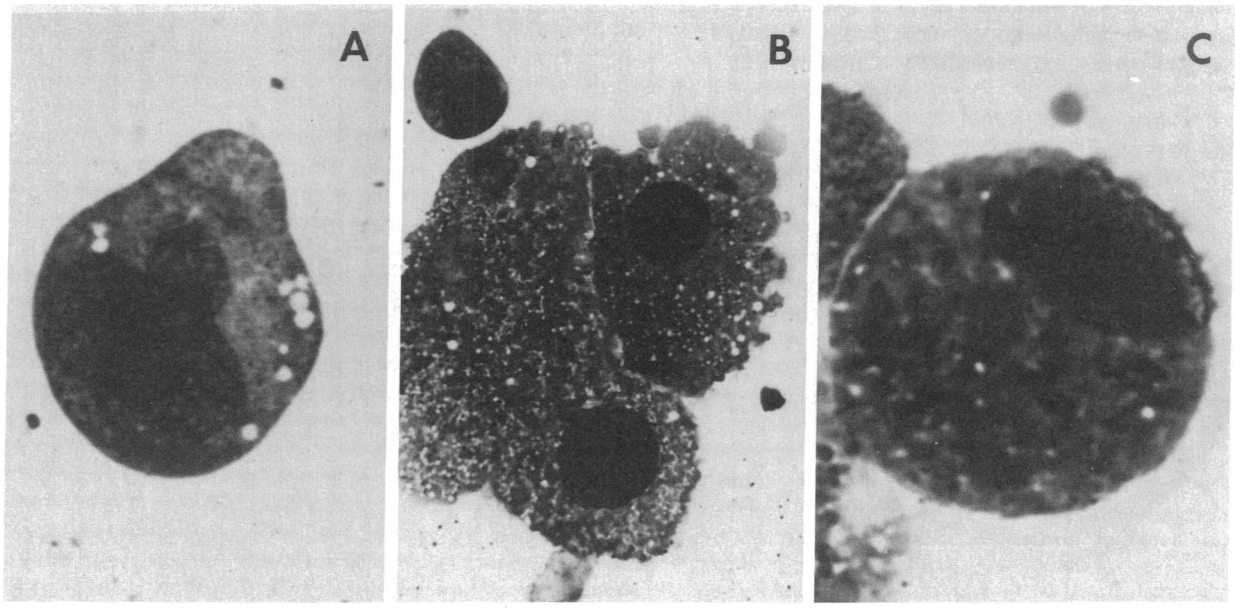

Figure 4. Cytospin preparations of representative cells from Wright-Giemsa stained RA synovial fluid-stimulated normal macrophage-depleted NT cells. (*A*) Large blastlike cells. (*B*) Mature macrophages. (*C*) Cells containing large basophilic and orange granules consistent with eosinophil precursors.

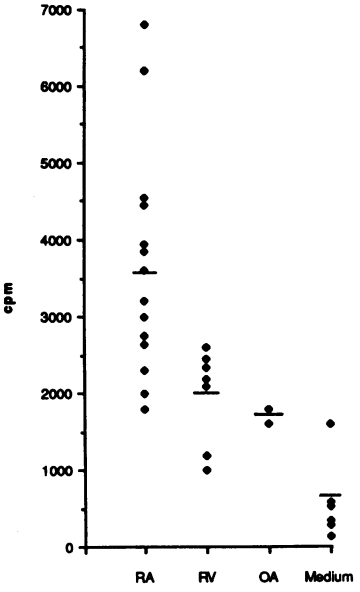

Figure 5. A comparison of CSF activity in SF from various joint diseases. Macrophage-depleted NT were cultured with medium alone or medium plus 5% SF and proliferation ([^{3}H]-Tdr) was measured after 15 d. Each point represents the mean of four determinations on the same sample using different sources of macrophage-depleted NT cells. (*RA*) Rheumatoid arthritis; (RV) rheumatoid variant (psoriasis, ankylosing spondylitis, Reiter's syndrome); (OA) osteoarthritis.

pendent. The proliferating cells in liquid cultures stimulated with synovial fluid were also similar to those seen in CSF-stimulated, liquid human marrow cultures, and contained eosinophil precursors and macrophages (7). In the present studies, undifferentiated blastlike cells, which have been grown from human blood and bone marrow (12), were also observed. Thus, both the proliferative responses and the morphology of the cells grown in liquid culture suggest strongly that synovial fluids contain CSF-like activity. Evidence that the responsible factor in synovial fluid is GM-CSF is its molecular mass (20–24 kD) and the ability of specific antibodies to GM-CSF to eliminate the CSF activity from synovial fluid.

Our experiments also demonstrate the production of biologically and immunologically active GM-CSF by RA synovial cells. Surprisingly, the RIA did not detect GM-CSF in synovial fluid samples, despite the presence of biologic activity. Several explanations were considered. Most likely is that the RIA is

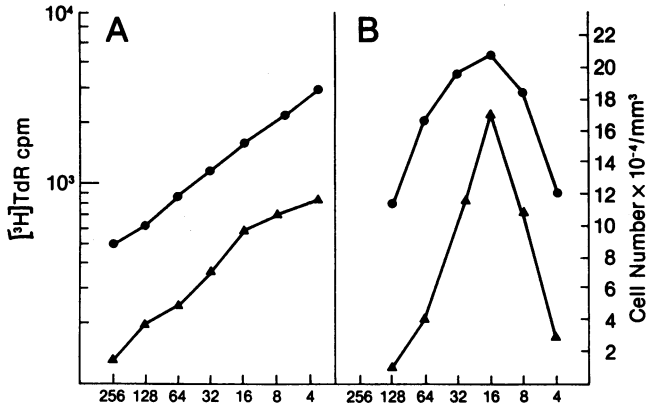

Figure 6. Representative studies of the effect of various dilutions of individual RA SF on macrophage-depleted NT cells (1×10^6/ml) from a normal donor. Viable cells (▲ —— ▲) and [^{3}H]Tdr uptake (● --- ●) were determined on day 15. *X*-axis shows reciprocal of the dilution of synovial fluid sample.

Table III. CFU-GM Assay of Synovial Tissue Explant Supernatants

Test sample	Day 7*	Day 14
Medium	0	0
rGM-CSF 10^{-9} M	19±4	35±8
PCM‡	248±46	179±13
ST 1	275±19	91±11
ST 2	117±17	121±8

Abbreviation: ST, synovial tissue.
* Mean±SEM colonies/10^5 cells in triplicate cultures.
‡ Placenta-conditioned medium.

relatively insensitive compared to the two bioassays used. In addition, small amounts of GM-CSF might be detected in bioassays through synergy with other factors known to be present in SF, such IL-1, CSF-1, or IL-6. Another interesting possibility, for which there is some preliminary data, is that inflammatory joint fluids cause an alteration in the epitopes of GM-CSF recognized by the monoclonal antibodies used in the immunoassay. These are not necessarily the antigens that react with the polyvalent antibody used in the neutralization experiments. In fact, some RA SFs that were negative in the RIA had demonstratable GM-CSF when measured in an ELISA employing another rabbit polyclonal anti GM-CSF antibody (unpublished data).

GM-CSF is not found exclusively in RA SFs. Similar biologic activity was also detected in effusions from other forms of inflammatory arthritis, albeit at a significantly lower level. This is consistent with earlier observations that the profiles of a variety of other cytokines are similar among the different forms of chronic inflammatory arthritis (3, 5).

In addition to identifying GM-CSF activity in SF, evidence of an inhibitor was also noted. This conclusion was based on the observation that some samples required substantial dilution in order to observe peak activity in the bioassay. Other cytokine inhibitors have been previously demonstrated in RA SF (3, 13); an IL-1 inhibitor produced by macrophages is the best characterized (13). Such factors may represent autoregulatory feedback loops that attenuate the synovial immune response.

Table IV. GM-CSF Radioimmunoassay

	GM-CSF
	*U/ml**
Medium	<10
ST 3	50
ST 4	140
ST 5 ADH‡	40
ST 5 NAD	40

Abbreviations: ST, synovial tissue supernatant; ADH, adherent cells; NAD, nonadherent cells.
* 10 U = 100 pg rhGM-CSF.
‡ ADH and NAD were separated on day 0 and supernatants from day 3 were assayed.

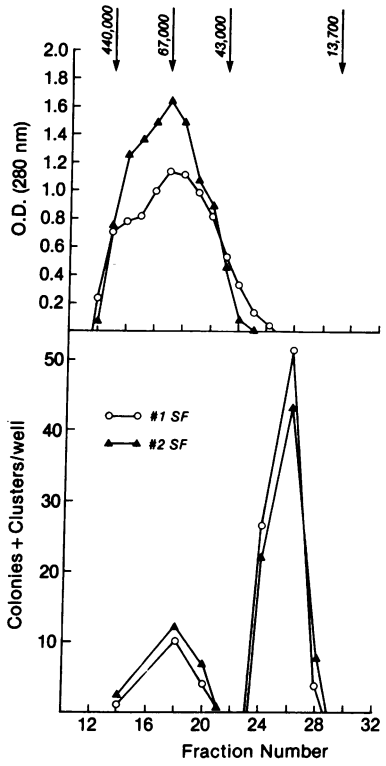

Figure 7. Sephadex G100 elution profile of two individual RA synovial fluids. (*Top*) Protein content (OD 280 nm) and (*bottom*) aggregate (colony/cluster) inducing activities on normal human macrophage-depleted NT population are shown. All values represent means of duplicate determinations. 2×10^5 cells were cultured in 96-well plates with a 1:5 dilution of each fraction for 15 d. Cellular aggregates were counted by inverted phase microscopy. Molecular mass markers shown are human ferritin 440 kD, bovine serum albumin (BSA) 67 kD, ovalbumin (OVA) 43 kD, and RNase A 13.7 kD.

Although RA SF is generally thought to contain a complex mixture of mediators, in reality, the cytokine milieu is restricted. For instance, T cell lymphokines like IL-2, IL-3, and IFN-γ (3, 5) are either absent or present in very low concentrations, while macrophage products like IL-1, tumor necrosis factor, and CSF-1 are abundant. Therefore, the identification of a potent immunomodulator like GM-CSF at the site of a chronic inflammatory disease is even more striking. This observation is also unique because GM-CSF has not previously been implicated in human disease.

Table V. Anti-GM-CSF Antibody Neutralizes Non-T Cell Proliferating Activity

Reagents	Anti-GM-CSF antibody	Cell count	[^{3}H]Tdr
		cells/mm^3	*cpm*
GM-CSF	−	$11.1 \times 10^4 \pm 2.8$	4,421±1,699
GM-CSF	+	$0.3 \times 10^4 \pm 0.6$	370±129
RA SF1	−	16×10^4	5,891
RA SF1	+	1×10^4	451
RA SF2	−	10×10^4	2,572
RA SF2	+	0×10^4	427
RA SF3	−	15×10^4	3,715
RA SF3	+	0×10^4	288

Macrophage-depleted NT cells from a normal donor cultured with GM-CSF (40 U/ml) or RA SF (5%). Rabbit anti-human GM-CSF antibody (1:50) was added at initiation of culture. Cell number and [^{3}H]Tdr were determined on day 15. The data for GM-CSF and GM-CSF plus antibody were the means of three donors.

The inflammation and structural damage characteristic of rheumatoid arthritis are thought to reflect two discrete, but interrelated processes. Immune complexes, interacting with polymorphonuclear leukocytes are responsible for the former; whereas products of the synovial lining cells likely cause disruption of articular structures. These synoviocytes have two derivations. The type A lining cells are of a macrophage lineage, while type B synoviocytes represent mesenchymal fibroblasts. Under the influence of IL-1 and other factors in the rheumatoid microenvironment, they produce large quantities of collagenase and prostaglandins. At the same time their surfaces show an enhanced expression of products of the class II major histocompatibility genes (Ia antigens). GM-CSF likely augments both the inflammatory and destructive elements of synovitis. By retaining granulocytes in the joint cavity (14) and expanding their inflammatory potential (15–17) GM-CSF may act to intensify or prolong local inflammatory reactions. GM-CSF might also participate in a paracrine interaction between adjacent synovial lining cells. Consider the following paradigm: Macrophages produce IL-1; IL-1 causes an enhanced elaboration of GM-CSF from fibroblasts (18); and GM-CSF is known to augment IL-1 production from monocytes and macrophages (6, 19). Thus, type A and type B synoviocytes could be constantly stimulating one another. Moreover, we have recently shown that GM-CSF is a potent inducer of HLA-DR expression on human monocytes and that antibody to GM-CSF neutralizes a non gamma interferon macrophage activating factor produced by RA synovial tissue cells (J. M. Alvaro-Gracia, N. J. Zvaifler, and G. S. Firestein, manuscript submitted for publication).

The origin of GM-CSF in synovitis is not known, although it can be synthesized by many cell types, including endothelial cells, fibroblasts, macrophages, and activated lymphocytes (18, 20–22). Our data from separated synovial tissue populations suggests that several different cells may be involved; however, on a per cell basis the adherent cells (primarily fibroblasts and macrophages) produce significantly more than the nonadherent cells (primarily lymphocytes). The identification of the source of GM-CSF in rheumatoid synovitis will be of great importance, both in terms of understanding the pathogenesis of the disease and in designing potential therapeutic interventions.

Acknowledgments

The authors thank Denise Smith for secretarial assistance and Michael Honeysett and Raymond Breen for technical assistance.

This work was supported in part by grants AM-14916, AR-07062, CA-32094, and AR-39576 from the National Institutes of Health.

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