Colony stimulating factor occurs in both inflammatory and noninflammatory synovial fluids

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Received July 23, 1989/Accepted February 6, 1990

Summary. Synovial fluids (SF) from patients with osteoarthritis (OA) and rheumatoid arthritis (RA) and various other arthritides were examined for the presence of colony stimulating factors (CSF). CSF was found in 7 of 13 (54%) SF from OA patients and in 8 of 12 (67%) SF from RA patients. It was also found in SF from patients with other arthropathies including 5 of 5 samples from patients with septic arthritis. Inhibition studies employing monospecific antisera indicated that in both RA and OA, CSF was of the macrophage type (M-CSF). While CSF was found in both inflammatory and noninflammatory effusions, significantly greater numbers of colonies were stimulated by RA SF than by OA SF and in general greater numbers of colonies correlated with higher SF leukocyte counts. Our data suggest that CSF as well as other cytokines may be involved in the perpetuation of joint destruction that occurs in various rheumatological conditions.

Key words: Colony stim factor – Arthritis – Synovial fluid

Introduction

The role of synovial fluid (SF) cytokines in the development and perpetuation of arthritis is currently a subject of intensive investigation. These mediators of immune and inflammatory responses could play active roles in arthropathy whether the initial insult is mechanical, immunologically mediated, or due to an infectious process. Interleukin-1 (IL-1) is the mediator most extensively studied in SF. It not only plays an integral role in T cell activation [1] but has chemotactic properties which might abet an inflammatory response [2, 3]. It can activate collagenases and proteoglycanases [3–7] and stimulate production of prostaglandins and plasminogen activators [5, 8]. All of these could contribute to cartilage destruction. Several studies have shown that SF samples from patients with various forms of arthritis contain IL-1 [2, 4, 9–11]. Small amounts of tumor necrosis factor-alpha (TNFα) which exhibits many biological activities that are similar to those of IL-1 have also been reported to occur in SF [12]. Colony stimulating factors (CSF), in addition to supporting the outgrowth and functional differentiation of various cell lineages from stem cells in the bone marrow, also exhibit powerful chemoattractant properties [13] and thus could influence the development of arthritis. Williamson et al. [14] have reported the presence of uncharacterized CSF in SF from several patients with rheumatoid arthritis (RA), psoriatic arthritis (PA), and in one patient with osteoarthritis (OA). Firestein et al. [15] have demonstrated colony stimulating factor (CSF) of the macrophage type in SF from patients with chronic inflammatory arthritis along with a factor that stimulates growth of mast cells.

In this paper we report the presence of CSF in SF samples from patients with both inflammatory and noninflammatory synovial effusions. The type of CSF present has been defined with specific antisera and the relative quantity of CSF in SF estimated by the number of colonies formed in agar.

Methods

Patients and synovial fluid collection: The diagnosis of classical or definite RA was made according to criteria set forth by the American Rheumatism Association [16]. Osteoarthritis was diagnosed on the basis of clinical history and examination, normal CBC, ESR below 40 mm/h and compatible X-ray findings. Other diagnoses were based on established clinical and laboratory guidelines. With the exception of patients with septic arthritis, nearly all patients whose SF was studied were being treated with antiinflammatory medications and approximately one-half of the RA patients were receiving therapy with disease-modifying antirheumatic drugs. Synovial fluid sent to our laboratory for analysis or aspirated for therapeutic purposes was centrifuged at 10000 rpm for 30 min and supernatants were stored in 1- to 2-ml aliquots at (-)70°C until tested.

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Colony Stimulating Factor Assay: This was done using a twolayer gel technique in 35-mm Petri dishes similar to the assay used by Williamson et al. [14]. The bottom layer consisted of 1.8 ml of 0.5% Seakem agarose (FMC, Rockland, Me., USA) and contained 20% fetal bovine serum, 35% Dubecco modified eagle medium (Sigma) in distilled water containing 40% DMEM, 20% FBS, and 100,000 murine bone marrow cells flushed from the femurs of $C_{57}BL/10$ mice (Jackson Laboratories, Bar Harbor, Me., USA). After 7–10 days at 37 °C in a humidified atmosphere containing 5% CO$_2$, colonies were counted, plucked with a pasteur pipet, washed, concentrated by cytocentrifugation (Shandon, Pittsburgh, Pa., USA) and stained with hemotoxylin and eosin (HE) for identification of cell types.

Inhibition studies were done by including specific rabbit antisera to human M-CSF, granulocyte-CSF (G-CSF), and granulocyte/macrophage-CSF (GM-CSF) at final dilutions of 1:100 in colony assays. Controls included rabbit anti-tumor necrosis factor (alpha) (TNF$\alpha$) and normal rabbit serum (NRS). All blocking and control reagents were obtained from Genzyme (Boston, Mass., USA).

Statistical analysis: Analysis of data was done using a computer program (True Epistat, Traci Gustafson, Richardson, Tex., USA). Student’s $t$-test was used to compare the number of colonies stimulated by SF from the various groups.

**Results**

CSF activity was found in 25 of 43 SF samples tested (58%). Examination of HE stained slides from colonies stimulated by RA, OA, and septic joint effusions revealed a predominance of cells having morphologic characteristics of macrophages. Occasional cells with a polymorphonuclear morphology were observed but these never comprised more than a few percent of cells recovered. The occurrence of CSF in SF is shown in Table 1. Seven of 13 (54%) samples from OA patients and 8 of 12 (67%) samples from RA patients contained CSF. All 5 SF samples from patients with septic arthritis contained CSF activity. CSF was sought in SF from patients with other diseases as well. These included patients with gout (1 of 4), psoriatic arthritis (1 of 2), Reiter’s syndrome (2 of 2), scleroderma (1 of 1). CSF was not found in SF from 4 patients with polyarthritis of uncertain etiology.

Table 1 also shows that the mean number of colonies stimulated by SF was significantly greater in septic arthritis compared with RA (140 vs 74 per 10$^6$ cells cultured; $P<0.05$) and in RA compared with OA (74 vs 19; $P<0.03$). Nine CSF negative samples were tested for the presence of inhibitors by adding them to a concentration of L929 supernatant that consistently yielded about 200 colonies and no sample was found to inhibit colony formation (data not shown).

Table 2 shows a representative experiment indicating the effects of various antisera on colony formation. SF exhibiting high CSF activity were purposely chosen for these experiments. CSF activity of SF from RA and OA patients were tested in the presence of NRS and rabbit antisera. Antiserum to M-CSF resulted in striking inhibition of colony formation in both RA and OA SF. Antisera to G-CSF, GM-CSF, and TNF$\alpha$ did not affect colony formation induced by OA SF but did partially inhibit the response caused by RA SF.

The relation between SF WBC count and number of colonies formed is shown in Table 3. In general higher SF WBC counts were associated with greater CSF activity. Samples with fewer than 1000 cells/mm$^3$ stimulated an average of 20 colonies while samples containing between 1000 and 10,000 cells/mm$^3$ stimulated an average of 95 colonies ($P=0.02$). SF containing more than 10,000 WBCs stimulated an average of 74 colonies per 10$^6$ marrow cells cultured ($P=0.06$). There was not a linear relationship between the number of colonies formed and the SF WBC count. The data in Table 3 show in addition that in the three categories of SF based on WBC count approximately the same fraction (about 66%) contained CSF activity.