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**Research Article**

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# Induction of Arthritis by Purified Cell-Derived Chemotactic Factor

## ROLE OF CHEMOTAXIS AND VASCULAR PERMEABILITY

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**ABSTRACT** The injection of monosodium urate-induced chemotactic factor into the joint cavities of rabbits induces an acute inflammatory response that resembles the one produced by monosodium urate crystals. The leukocyte accumulation induced by the factor was not accompanied by a measurable increase in vascular permeability as measured by appearance of  $^{125}\text{I}$ -albumin in the joint cavity. When histamine was injected into the joints, a marked increase in vascular permeability but no leukocytosis above control levels was observed. The above results suggest that the cell-derived factor is primarily responsible for the accumulation of cells seen in the acute inflammation induced by monosodium urate crystals.

### INTRODUCTION

Studies performed in experimental animals suggest that the consistent requirement for the development of acute crystal-induced arthritis is the interaction of crystals and polymorphonuclear leukocytes (PMNs)<sup>1</sup> in the joint cavity (1-9). The ensuing phagocytosis of crystals by the cells results in the formation of a glycoprotein (mol wt 8,400) with chemotactic properties for neutrophils and mononuclear cells and the release of inflammatory substances from the cells during phagocytosis and (or) cell death (10-13). The present study was designed to test the ability of the purified monosodium urate-induced chemotactic factor to produce an acute inflammation *in vivo* comparable to the one obtainable with intraarticular in-

jection of monosodium urate crystals (3, 6) and to study the role that vascular permeability may play in the events that lead to intraarticular leukocyte accumulation.

### METHODS

*Preparation of chemotactic material.* Rabbit peritoneal neutrophils were obtained as described by Cohn and Hirsch (14). The preparation was 90% neutrophils and the contaminating cells were mainly erythrocytes. The chemotactic material was prepared as follows: approximately  $30 \times 10^6$  rabbit peritoneal neutrophils were incubated at 37°C for 45 min in 5 ml of isotonic saline with 3 mg of synthetic monosodium urate crystals made pyrogen free by heating at 200°C for 2 h (4). The cells were disrupted and the lysosomal fraction obtained as previously described (12). The lysosomal fraction of the cell was then disrupted by ultrasonication for 45 s on ice, and centrifuged at 20,000 g for 20 min in a Beckman model L Ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The  $\beta$ -galactosidase activity was 1,058.4 pmol of umbelliferone release per 100  $\mu\text{g}$  protein/h in the lysosomal fraction as compared to 132.3 in the whole cell lysate. The lysosomal fraction was passed over a  $63 \times 1.5\text{-cm}$  G-50 Sephadex column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in isotonic saline solution and the fraction containing the chemotactic activity (0.11 of eluate volume) from several experiments was pooled, dialyzed against deionized water, lyophilized, and run (50  $\mu\text{g}$ ) in preparative polyacrylamide gel electrophoresis in glycine buffer (0.38 M glycine and 0.005 M Tris), pH 8.3, at 2 mA per tube (15) for 1 h. The gels measuring  $5 \times 75$  mm were stained with Coomassie Blue (16) for protein and for carbohydrate by the method of Zacharius et al. (17). The lowest amount of protein and carbohydrate that we can visually detect with the above methods is 1  $\mu\text{g}$  for protein and 2  $\mu\text{g}$  for carbohydrate. Unstained gels were sliced in equal fractions and their contents eluted with distilled water. The eluates were then tested for chemotactic activity (12). All studies were performed utilizing the eluate of the gel slice containing a single chemotactic line (13). The preparation of the nonchemotactic control follows the same procedure but cells were killed by freezing before incubation with the crystals.

*Synovial inflammation.* 20 small (1.5 kg) albino rabbits were injected with 20  $\mu\text{g}$  of the purified chemotactic factor

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<sup>1</sup>Abbreviation used in this paper: PMN, polymorphonuclear leukocyte.

in isotonic saline (0.3 ml) in one knee and with isotonic saline in the opposite knee; and 8 more rabbits were injected with 10 mg of synthetic monosodium urate crystals suspended in saline in one knee and isotonic saline in the opposite one. Two parameters were employed to assess the inflammatory response: histological evaluation of the synovium and leukocyte counts in the synovial fluid. Animals were killed at different intervals, from 1 to 4 h, after the intraarticular injections of the chemotactic factor and up to 5 h for monosodium urate crystals. Synovial tissue samples were stained with hematoxylin and eosin and samples were cultured in broth and blood agar plates to exclude the possibility of bacterial contamination. The histological preparations were evaluated blindly. Leukocyte counts in the synovial fluid were performed immediately with a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.) (7). In a separate set of experiments, 12 animals were injected intravenously with 25  $\mu$ Ci of  $^{125}$ I-albumin (sp act 1.0  $\mu$ Ci/mg; Mallinckrodt Inc., St. Louis, Mo.) immediately followed by intraarticular injections of chemotactic factor and control in opposite knees. The controls used were as follows: isotonic saline, histamine (50  $\mu$ g), and synthetic monosodium urate crystals (10 mg). The last two agents were used as positive controls in the experimental design. At the end of 90 min the animals were sacrificed and their synovial fluid collected and counted for radioactivity with the aid of a gamma counter and leukocyte counts obtained. Results are expressed as radioactive counts per min  $\pm$  SD and leukocyte counts per cubic millimeter.

## RESULTS

An acute inflammatory response manifested by intense leukocyte accumulation in the synovial fluid and synovial membrane was observed after the injection of monosodium urate-induced chemotactic factor into the joints of the rabbits. The intense leukocytosis in the synovial fluid was evident at 60 min, reached its peak at 90 min, and was followed by a gradual decrease during the time span of the experiment. The differential count in the synovial fluid showed 95% neutrophils and 5% mononuclear cells. The synovial fluid obtained from saline control joints showed minimal leukocytosis (Fig. 1). The histological evaluation of the synovial membrane from joints injected with the chemotactic factor demonstrated an inflammatory infiltrate composed of mainly mononuclear cells and to a lesser degree neutrophils (Fig. 2). The synovial tissue from control joints appeared normal. The synovial fluid leukocytosis induced by monosodium urate crystals, although evident at 90 min, increased markedly with time, the counts  $\pm$  SD per  $\text{mm}^3$  at 90 min were 19,247  $\pm$  6,073, at 180 min 134,039  $\pm$  22,006, and at 300 min 319,429  $\pm$  11,001. The synovial tissue showed an intensive infiltrate with mononuclear cells, and neutrophils to a lesser degree.

When the rabbits were injected intravenously with  $^{125}$ I-albumin immediately preceding the intraarticular injection of chemotactic factor and control in contralateral knees, no difference in the radioactive counts between the knees injected with the chemotactic factor and with saline was observed at the end of 90 min. The

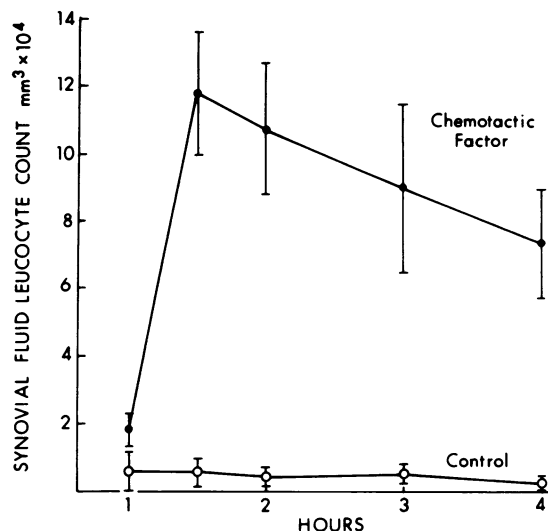


FIGURE 1 Synovial fluid PMN leukocytes after injection of urate-induced chemotactic factor. Bars represent SD.

counts per minute in the knees injected with the positive controls histamine and monosodium urate crystals were about nine times greater than for saline and for the chemotactic factor. The leukocyte count in the synovial fluid of the rabbits injected intraarticularly with the chemotactic factor was, however, about 17 times greater than for saline or histamine and about 6 times greater than for urate crystals at 90 min (Table I).

## DISCUSSION

Intraarticular monosodium urate crystals have been documented to be the causative agent in the acute gouty attack of man as well as experimental arthritis in various animals (1, 2). Although a number of soluble factors have been proposed to mediate the inflammatory reaction (2), studies performed in experimental animals have shown that crystal-induced arthritis can occur independently of complement activation (3–5), kinin generation (6, 7), and Hageman factor activity (7). It is not possible, however, to elicit a crystal-induced arthritis in neutropenic animals (5, 8, 9). Although evidence is still incomplete, the above studies suggest that the only consistent requirement for the development of the acute monosodium urate crystal arthritis is the interaction of crystals with the PMNs normally found in the synovial space. The ensuing phagocytosis of crystals results in the formation of a glycoprotein chemotactically active for neutrophils and, to a lesser degree, mononuclear cells (10–13), and leads to the release of lysosomal proteases and other inflammatory substances from the cell due to exocytosis and (or) cell death. If this concept approximates the events leading to the development of the acute inflammatory response to monosodium urate crystals, the introduction of the

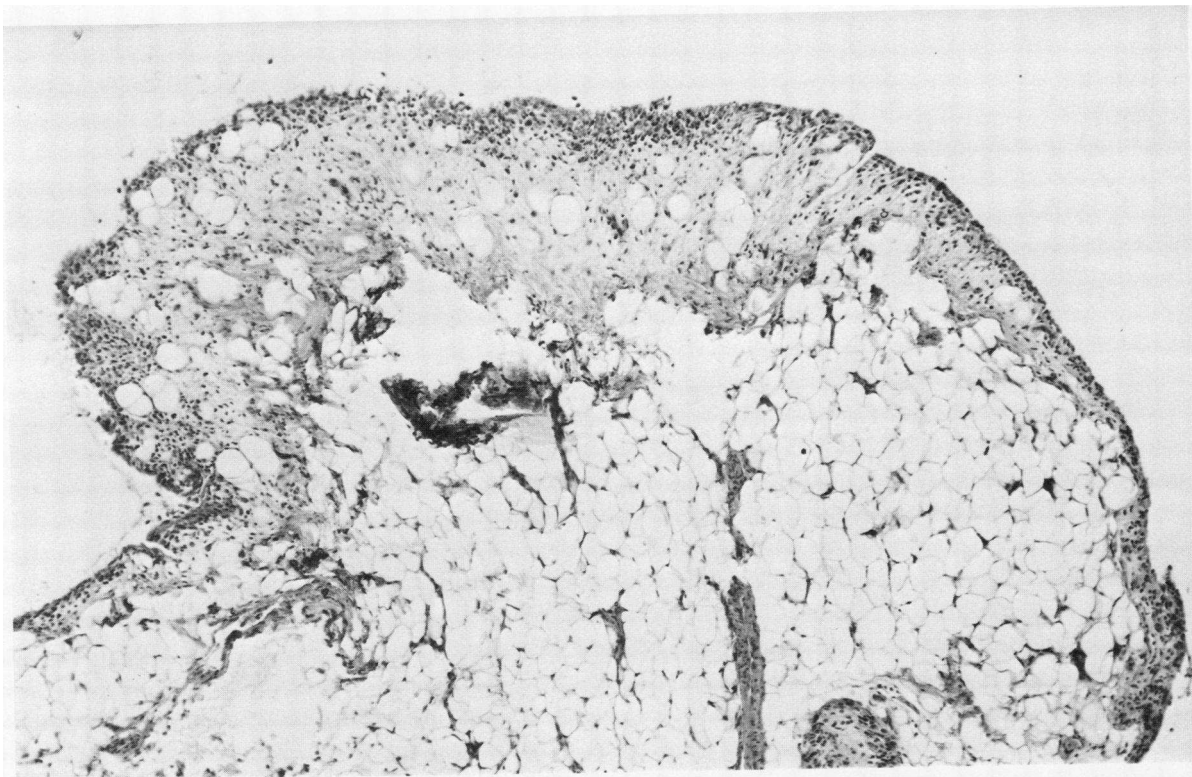


FIGURE 2 Rabbit synovium 2 h after injection of urate-induced chemotactic factor. Marked mononuclear infiltrate is present (magnification  $\times 400$ ).

chemotactic factor into the joint cavity should lead to an inflammatory response analogous to the one seen in acute crystal-induced inflammation. The present experiments indicate that the intraarticular injection of urate-induced chemotactic factor into the joint cavity of rabbits induces an inflammatory response that closely resembles the one obtained in experimental models of monosodium urate crystal arthritis (3, 6, 8),

as well as acute human gout. The peak of the response, however, appears earlier, since the first stage of the inflammatory process, the meeting of cells and crystals leading to phagocytosis and generation of the chemotactic factor, had taken place in vitro in our experimental design. Our experiments also suggest that the inflammatory infiltrate seen in the rabbit joints were the results of the chemotactic properties of the factor upon the cells and not due to an increase in vascular permeability, as measured by the appearance of  $^{125}\text{I}$ -albumin in the joint fluid. The rabbit joints injected with histamine showed an increase in vascular permeability, with a mild leukocytosis not greater than the saline control, at 90 min, suggesting that vascular dilatation plays a minor role in the accumulation of leukocytes in the acute inflammatory response. Monosodium urate crystals also induced vascular dilatation in the rabbit joint but the degree of leukocytosis at 90 min was considerably less than the one elicited by the chemotactic factor, this leukocytic effect of urate crystals was probably mediated mainly by urate-induced chemotactic factor beginning to be generated and released from the neutrophils. Leukocytes contain enzymes that both produce and destroy kinins (18, 19), mediators of vascular dilatation; since vascular dilatation is seen in the inflammatory process induced by

TABLE I  
Degree of Leukocytosis and Vascular Permeability (as Measured by Appearance of  $^{125}\text{I}$ -Albumin in the Joint Cavity) Induced by Injection of Neutrophil-Derived Chemotactic Factor and Controls

	cpm* $\pm$ SD	WBC $\times$ mm <sup>3</sup> $\pm$ SD †
Chemotactic factor, 20 $\mu$ g	172.8 $\pm$ 70	118,426 $\pm$ 47,556
Isotonic saline	152.4 $\pm$ 56	6,965 $\pm$ 5,936
Monosodium urate crystals, 10 mg	1,655.7 $\pm$ 1,203	19,247 $\pm$ 6,073
Histamine, 50 $\mu$ g	1,526.6 $\pm$ 516	6,883 $\pm$ 2,947

Results are expressed as mean  $\pm$  SD of 12 rabbit knee joints for the chemotactic factor and 4 for each control. Animals were sacrificed 90 min after joint injections.

\* Radioactive counts per minute.

† WBC, leukocytes.

urate crystals, it is likely that kinins and perhaps other unidentified substance(s) play a supportive role in the development of the inflammatory process.

In summary, our studies demonstrate that intraarticular injection of purified monosodium urate crystal-induced chemotactic factor can elicit a profound leukocytic accumulation in the joint cavity without demonstrable change in vascular permeability. This finding supports the premise that the sole requirement for the development of the acute gouty attack is the phagocytosis of the monosodium urate crystals by the neutrophil, and possibly other phagocytic cells.

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