HUMAN MONONUCLEAR CELLS AND NEUTRAL PROTEINASES

III. Neutral Proteinases and Rheumatoid Arthritis: Monocytes as a Source of Cathepsin G and Proteinase Potentiation of IgM Rheumatoid Factor Elaboration

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Abstract—We have been interested in contributions of certain cells and mediators to synovial inflammation rheumatoid arthritis (RA). The present studies were designed to determine (1) whether monocytes contained the neutral proteinase cathepsin G and (2) if neutral proteinase could induce or potentiate cellular IgM rheumatoid factor (RF) production. Monocyte-rich and monocyte-poor populations were isolated by Ficoll-Hypaque density sedimentation followed by glass adherence, and cellular lysates were obtained by repetitive freezing and thawing as we have reported for neutrophil-derived neutral proteinase. Cathepsin G was quantified immunologically by an enzyme-linked immunosorbent assay (ELISA) we developed utilizing commercially available anti-cathepsin G antibodies. Mononuclear and B-cell-enriched cell cultures were prepared by standard methods and IgM RF measured by our ELISA. Cell-derived lysates from monocyte-enriched populations (84 ± 3% monocytes, less than 1% neutrophils) contained considerably greater amounts of measurable cathepsin G (OD280 = 0.393 ± 0.153) than lysates from equal numbers of monocyte (15 ± 2% monocytes, less than 1% neutrophils) -depleted cells (OD280 = 0.071 ± 0.038; P < 0.05). Eighteen patients with RA and three normal individuals did not have consistently increased cellular elaboration of Ig or IgM RF in vitro in response to proteinase (trypsin) stimulation, however, patients manifested 80% potentiation by trypsin of pokeweed-stimulated cellular IgM RF production in vitro (pokeweed-stimulated IgM RF 137 ± 55 ng/ml, pokeweed/trypsin-induced IgM RF 246 ± 100 ng/ml; P < 0.02), changes being most striking for those patients seropositive by

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latex fixation test (84% increase, \( P < 0.02 \)). These data indicate that monocytes and/or macrophages localized to sites of inflammation are a source of cathepsin G and that neutral proteinases may potentiate cellular activation and auto antibody production in processes such as rheumatoid arthritis.

**INTRODUCTION**

We have been interested in the interrelationships between neutrophilic and mononuclear cells as they pertain to the pathogenesis of rheumatoid arthritis (RA). We described neutrophil-derived factors that potentiated B-lymphocyte responses (1) and tentatively identified a human neutrophil-derived B-lymphocyte enhancing factor, N-LEF, as cathepsin G (2). In other experiments we have examined control of synovial cell IgM rheumatoid factor (RF) production by soluble mediators and cells (3, 4), particularly monocytes (5). The present studies were an extension of this work designed to determine whether: (1) monocytes contained cathepsin G, and (2) neutral proteinase could induce or potentiate cellular elaboration of IgM RF in patients with RA.

**MATERIALS AND METHODS**

*Human Subjects.* Blood was collected and processed from eight healthy persons using no medications and from 17 patients fulfilling American Rheumatism Association (ARA) criteria for definite or classical RA, not all of whom could be studied in each experimental situation. Patients underwent clinical, radiologic, and laboratory evaluation as previously described (1-5). These studies were approved by institutional review boards.

*Cell Cultures.* We collected heparinized blood and, when available, synovial fluids (SF) from patients. Mononuclear cells (MNC) were obtained by Ficoll-Hypaque density gradient separations, as described (1-5). For certain experiments, MNC were further separated into monocyte-enriched and depleted preparations by sedimentation on gradients of 40-53% Percoll, 286 mosm/kg, as described (5). T, B, and monocyte populations were identified by sheep red blood cell rosettes, surface immunoglobulin, and peroxidase staining and morphology, respectively, as we have reported (3-5). Certain cell lines (IM 9 (B cells), RPMI 7666 (T cells) and CEM (T cells]) were used as we have described (6, 7). Optimal culture conditions for enzyme linked immunoassays (ELISA) were 5 \( \times \) 10^5 cells/ml in RPMI 1640, with 10% fetal calf serum, with or without pokeweed mitogen (PWM) (1:100). Cellular supernatants were obtained and analyzed for IgG, IgA, IgM, and IgM RF content after 12 days, as described (3-5).

*ELISA.* Culture supernatants were assayed by the ELISA technique we reported in detail (3). For IgM RF, plates were coated with heat-aggregated (63°C, 10 min), DEAE-purified, Cohn fraction II IgG, 10 μg/ml. Samples were diluted, incubated, and assayed as for Ig or with buffer adjusted to pH 4.1. IgM RF standards were prepared from RF-positive sera by sequential globulin precipitation, Sephacryl-S300 gel filtration, and IgG-Sepharose affinity chromatography as we have related (4, 5).