STIMULATED RELEASE OF NEUTRAL PROTEINASES ELASTASE AND CATHEPSIN G FROM INFLAMMATORY RAT POLYMORPHONUCLEAR LEUKOCYTES

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Abstract—Rat leukocytes from inflammatory peritoneal exudates respond in vitro to a variety of chemotactic and phagocytic stimuli by releasing both elastase and cathepsin G neutral proteinase enzyme activities. PAF, FMLP, and PMA stimulated a rapid, cytochalasin B-dependent, dose-related release of both enzymes; however, leukotriene B_4 was inactive. It was not possible to measure the activity of zymosan-activated serum on these cells as rat serum contains high levels of proteinase inhibitors. The calcium ionophore A23187 stimulated a dose-related, time-dependent, cytochalasin B-independent enzyme release. Concanavalin A stimulated a weak, non-dose-related release of enzyme activity. Zymosan and serum-coated zymosan stimulated enzyme secretion which was markedly inhibited by the presence of cytochalasin B. These data indicate that release of azurophilic granule neutral proteinases from rat inflammatory leukocytes can be detected and quantitated in vitro. This model could provide a test system for monitoring the pattern and specificity of enzyme release from azurophil granules. The ability of a variety of stimuli to induce proteolytic enzyme release from inflammatory neutrophils may be of considerable relevance to chronic inflammatory diseases.

INTRODUCTION

Polymorphonuclear leukocytes (PMNs) have long been recognized as important components of acute inflammatory reactions in terms of both phagocytosis with enzymic degradation of microorganisms and extracellular secretion of proteolytic enzymes thought to be involved in the tissue-destructive manifestations of inflammation (1, 2). Much of the work on PMN granular enzymes has concentrated on the human peripheral blood PMN; however, recently some studies have examined the enzymic arsenal of rat inflammatory PMNs (3, 4). A study of rat PMN enzymes is of some relevance as, firstly, the rat is the predominant animal model used to study experimental arthritis (5), and secondly, it allows
for the study of acute inflammatory PMNs which may be more relevant to tissue inflammation than the circulating PMNs. Recent studies have demonstrated the presence in rat PMNs of enzymes from both specific and azurophilic granules, including the neutral proteinases elastase and cathepsin G. These enzymes are of particular interest in view of their ability to cleave a wide range of proteins including virtually all connective tissue components, thus implicating them in a variety of pathological conditions (6–9).

A considerable number of reports have demonstrated that a variety of extracellular agents can stimulate PMN degranulation, including chemotactic factors such as the bacterial peptide analog N-formyl-methionyl-leucyl-phenylalanine (FMLP) (10, 11), the complement component C5a (12), and the membrane phospholipid products 5-hydroxyeicosatetraenoic acid (5-HETE), leukotriene B4 (5,12-dihydroxyeicosatetraenoic acid), and the platelet-activating factor 1-O-acetyl-alkyl-glyceryl-phosphochline (PAF) (13–16). Also known to stimulate PMN degranulation are phagocytic stimuli such as zymosan (17) or antigen–antibody complexes (18) and membrane destabilizing agents such as calcium ionophores (19, 20) and the tumor-promoting phorbol esters (21).

We have therefore studied the release of rat peritoneal leukocyte elastase and cathepsin G in response to a variety of degranulating stimuli with a view to analyzing the pattern of azurophil granule enzyme secretion and the establishment of a reproducible and relevant in vitro test procedure for the study of potential inhibitors of inflammatory cell enzyme release.

MATERIALS AND METHODS

Preparation of Rat Leukocytes. Outbred Wistar rats (400–600 g; RA25; Sisseln, Switzerland) were injected intraperitoneally with 16 ml of a 12% w/v sodium caseinate suspension in physiological saline and sacrificed 18 h later. Peritoneal cells (>85% PMN) were removed, washed twice in phenol red-free Eagle’s minimal essential medium (E-MEM), buffered to pH 7.4 with 30 mM N’-2-hydroxyethylpiperazine-N’-2-ethane sulfonic acid (HEPES) and resuspended in the same buffer at a concentration of 1 × 107 viable PMN/ml (viability assessed by trypan blue dye exclusion as >95%).

Stimulation of Enzyme Release. Aliquots (0.5 ml) of cell suspension were placed into sterile plastic tubes (Sterelin, Teddington, U.K.) and prewarmed to 37°C in a shaking water bath for 10 min. Following addition of stimulus or vehicle, the cell suspensions were further incubated at 37°C for the times indicated in the results. Following incubation, the suspensions were rapidly centrifuged (10,000g) for 2 min at 4°C and the supernatants transferred to fresh tubes. Three 0.15-ml aliquots of each supernatant were prepared and stored at −20°C prior to assay for enzyme activity. In studies utilizing cytochalasin B, warmed cells were preincubated with the cytochalasin B for 10 min prior to addition of the chemotactic or phagocytic stimulus.

Assay for Rat Leukocyte Elastase (RLE) Activity. Samples of PMN culture supernatants were diluted 1:5 in 0.2 M Tris HCl buffer (pH 7.5) containing 25 μg/ml human serum albumin (Calbiochem, LaJolla, California) and 200 μl assayed with 0.2 ml of 2 mM MeOSucc-Ala-Ala-Pro-Val-pNA (Bachem, Bubendorf, Switzerland) in 0.2 M Tris as specific substrate (22). Samples were incubated at 37°C for appropriate times (0.5–4 h) and the reaction terminated by addition of