NEUTROPHILIC LEUKOCYTES IN IMMUNOLOGIC REACTIONS IN VITRO

IV. The Effect of Trypsin

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Abstract—Prior trypsinization of rabbit PMN prevented the normal selective release of lysosomal constituents induced by contact with zymosan-C3 and abolished the adherence of these cells to sheep RBC sensitized with IgM antibody and complement (PMN rosettes). The effect of trypsin could be completely reversed by exposure of the cells to soybean trypsin inhibitor after trypsinization. Trypsin did not inhibit the lysosomal release provoked by contact with immune complexes or interfere with rosette formation between PMN and sheep RBC sensitized with IgG antibody. The action of trypsin on the PMN C3b receptor may not be enzymatic.

INTRODUCTION

Neutrophilic leukocytes (PMN) appear to possess at least two distinct surface receptors which promote the attachment and phagocytosis of opsonized particles. One of the receptors binds the Fc portion of IgG; the second, the activated third component of complement, C3b (1–3). Evidence that the two receptors are distinct has come largely from two sources, one based on kinetic analysis of particle phagocytosis and the other derived from observations on the effect of trypsinization of PMN. Kinetic analyses have shown that the mechanisms of action of IgG (Fc) and C3b are different (1), that they may be synergistic, and, more recently, that particle-bound C3b is important primarily in the attachment phase but only bound IgG can initiate phagocytosis (3). The other studies supporting distinctness of these receptors demonstrated that prior trypsinization of PMN prevented their adherence to erythrocytes sensitized with IgM antibody and complement, but that the adherence to erythrocytes sensitized with IgG antibody alone was unaffected.

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(4). A similar observation has been made with respect to monocytes (2). These studies implied that the C3b receptor was a membrane ectoprotein susceptible to destruction by tryptic hydrolysis (2). We have recently observed that the effect of trypsin on several PMN functions in vitro is reversible, and thus its action in modifying PMN response to opsonized particles and immune complexes may be nonenzymatic. These studies form the basis of this report.

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: New Zealand White rabbits, Canadian Breeding Laboratories, St. Constant, Quebec; ferritin, Miles Laboratories Kankakee, Illinois; soybean trypsin inhibitor, Sigma Chemical Co., St. Louis, Missouri; zymosan, Fleischmann Laboratories, Standard Brands Inc., New York, New York; trypsin, Worthington Biochemical Corp., Freehold, New Jersey; IgG and IgM amboceptors, Maynard Diagnostics, Downsview, Ontario; sheep red blood cells, Armand Frappier Inst., Laval, Quebec; 51Cr (sodium chromate), Charles E. Frosst & Co., Montreal, Quebec.

Neutrophil Preparation. PMN were obtained from the peritoneal cavity of New Zealand White rabbits 4 h after injection of 50 ml of 0.1% glycogen in sterile saline. The resulting preparation contained more than 95% PMN. When necessary, the suspension was cleared of erythrocytes by hypotonic lysis as described previously (5). The final cell preparation was washed and suspended in Puck's buffered saline, pH 7.4, at a concentration of $2.5 \times 10^6$ PMN per ml.

Immune Complexes. Ferritin–rabbit antiferritin complexes in precipitate form were prepared as described previously (5).

Zymosan-C3. Zymosan-C3 was prepared as described previously (6). The final suspension contained 10^9 particles per 0.5 ml.

Neutrophil Lysosomal Constituent Release. The release of lysosomal constituents from PMN exposed to immune complexes or zymosan-C3 was measured as follows: $2.5 \times 10^6$ PMN in 2.5 ml of Puck's saline, pH 7.4, was pipetted into acid-cleaned glass petri dishes (55-mm diameter). The plates were covered, incubated for 20 min at 37°C, then washed once with ice cold Puck's saline. Monolayers prepared in this way contained 90% of the applied PMN. The monolayer could be washed at least four times with ice cold Puck's with loss of less than 5% adherent cells. PMN remained adherent to the glass and viable for more than 90 min at 37°C. This method has been employed in preference to free suspensions of cells because it gives more reproducible results and eliminates cell clumping. Monolayers so prepared were incubated with trypsin, 0.2% final concentration, in Puck's, pH 7.4 or pH 8.0, for varying times at 0°C, 22°C, and 37°C, then washed once with cold Puck's. Control monolayers were incubated in a similar fashion without trypsin. Selected preparations were then exposed to soybean trypsin inhibitor (SBTI) by adding 2.5 ml of 0.2% SBTI in Puck's, pH 7.4. These monolayers were incubated for 30 min at 37°C and washed once with cold Puck's. Control plates, trypsinized plates, and SBTI-treated plates were exposed to immune complexes or zymosan-C3 particles in a total volume of 2.5 ml of Puck's and incubated for 60 min at 37°C. The supernatant from each plate was decanted and centrifuged 350g for 10 min at 0°C.

Enzyme Assays. Monolayer supernatants and tritonized cell suspensions were assayed