INHIBITION OF HUMAN EOSINOPHIL CHEMOTAXIS BY IgA PARAPROTEINS

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Abstract—Sera from patients with IgA myeloma inhibit normal human eosinophil chemotaxis. No correlation was noted between inhibition and the absolute concentration of IgA or \(\lambda\)-k light-chain type. Eosinophil chemotactic inhibitory activity was associated with isolated IgA paraproteins and was found to be cell directed and stable at 56°C. Pepsin digestion of IgA paraproteins resulted in loss of both IgA Fc fragment and eosinophil chemotactic inhibitory activity. Polymeric IgA accounted for most of the inhibitory activity as evidenced by sucrose density gradient centrifugation studies and a loss of inhibitory activity following dithiothreitol reduction and iodoacetamide alkylation which converted polymeric IgA to monomeric IgA. Comparative studies with neutrophils showed that both neutrophil and eosinophil chemotaxis and chemokinesis were effectively inhibited by IgA paraproteins. The mechanisms of suppression of eosinophil and neutrophil chemotaxis by IgA paraproteins appear to be similar and possibly may involve a membrane receptor for IgA.

INTRODUCTION

Patients with myeloma show an increased susceptibility to bacterial infection and have been shown to have a decreased immune response (1, 2). Studies by Penny et al. indicate that patients with IgG or IgA myeloma show a marked decrease in neutrophil adhesiveness and phagocytosis (1). In the latter studies a direct correlation was observed between the serum myeloma component concentration and decreased phagocytosis. Ziegler and coworkers have examined the inflammatory response in patients with IgG or IgA myeloma and found that seven of eight patients with IgA myeloma and eight of twelve patients with IgG myeloma showed decreased in vivo inflammatory responses as measured by the Reubuck skin window technique (2). Recently, it was observed that sera from patients with IgA myeloma significantly suppressed normal neutrophil and monocyte chemotaxis in vitro (3). The chemotactic inhibitory activity in these samples was
found to be directly associated with the isolated IgA paraprotein; moreover, IgA inhibitory effects were cell directed, dependent on the Fc region of IgA, and associated primarily with polymeric forms of IgA. More recent studies indicate that IgA paraproteins suppress neutrophil bactericidal activity in a similar manner (4). Studies by Lawrence et al. (5, 6) on the cytophilic activity of human myeloma proteins have demonstrated a specific polymorphonuclear leukocyte receptor for IgA1, IgA2, and secretory IgA. Their data indicated that an intact IgA Fc fragment was necessary for cell membrane binding; reduction-alkylation of IgA paraproteins abolished binding, and binding was increased by aggregation of the respective IgA paraproteins. It seems possible that inhibition of neutrophil chemotaxis and bactericidal activity by IgA paraproteins (3, 4) may be the result of an interaction between IgA and cell membrane IgA receptors.

Since IgA paraproteins inhibit neutrophil chemotaxis to a greater extent than monocytes, the question of the specificity for the granulocytic series arose. The following study was undertaken in order to determine if IgA paraproteins were capable of suppressing the chemotactic response of human eosinophils.

MATERIALS AND METHODS

Eosinophil Preparation. Human eosinophils were prepared from the heparinized (10 units/ml) peripheral blood of healthy adult donors. Leukocyte-rich cell suspensions were obtained by adding 1 cc of Plasmagel (HTI Corp., Buffalo, New York) per 5 cc blood and allowing erythrocytes to sediment for 30 min at 37°C. Eosinophils were isolated from this suspension by density gradient centrifugation (45 min, 400 g) over a Hypaque cushion with a density of 1.144–1.148 according to the method of Day (7). The resulting pellet contained 50–99% eosinophils as determined by Wright-stained cell smears and were greater than 95% viable by trypan blue dye exclusion. Cells were washed, pooled, and suspended to a concentration of 5×10^6 eosinophils/ml in Hanks' balanced salt solution (HBSS) with 2% bovine serum albumin (HBSS-BSA).

Neutrophil Preparation. Human neutrophils were obtained from heparinized peripheral blood of healthy adult donors. Erythrocyte sedimentation was performed by the addition of Plasmagel (1 cc Plasmagel/5 cc blood), and the white cell suspension was diluted with HBSS and layered over a Ficoll-Hypaque solution (8). Neutrophils were separated by centrifugation at 400g for 45 min (3). The cell pellet containing greater than 90% neutrophils was washed and resuspended to a final concentration of 7×10^6 cells/ml in HBSS.

Isolation of IgA and IgG Paraproteins. IgA was prepared from the serum of patients with IgA myeloma containing greater than 1500 mg/100 ml of IgA by a combination of starch block electrophoresis and diethylaminoethyl cellulose (DEAE) ion-exchange chromatography (9). IgA paraproteins were adjusted to a concentration of 10 mg/ml, as determined by optical densities at 280 nm, and checked for purity by Ouchterlony immunodiffusion (10).

IgG paraproteins were prepared from myeloma sera by DEAE ion-exchange chromatography and Sephadex G-200 gel filtration chromatography (9). IgG was adjusted to a concentration of 10 mg/ml and checked for purity by Ouchterlony immunodiffusion analysis.