Ia Antigen is a Differentiation Marker on Human Eosinophils

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Evidence suggests that the "la-like" or DR antigen is a differentiation marker that is expressed on early human hematopoietic precursor cells, but is absent on their mature progeny. The eosinophil precursor cell (CFU-EO) is distinct from the granulocyte-monocyte colony-forming cell (CFU-C). We provide data that indicate that the Ia antigen is expressed on the human eosinophil colony-forming cells and is absent on mature eosinophils. All CFU-EO were inhibited in the presence of rabbit Ia antiserum at a titer of 1:30. Cytotoxicity was complement-dependent. The metamyelocytic eosinophil and more mature eosinophil forms did not express the Ia antigen.

HUMAN B LYMPHOCYTES express a cell membrane antigen (Ia-like) that is not found on unstimulated peripheral blood T lymphocytes. This antigen is a product of genes within the major histocompatibility region and is a membrane polypeptide dimer having molecular weights of approximately 27,000 and 35,000 daltons. The antigen is termed "Ia-like," or simply Ia antigen, because of its similarity to the murine I-region gene products. The Ia antigen is expressed on myeloid, erythroid, and B-lymphocyte progenitor cells. However, it is absent from mature blood cells such as neutrophils, erythrocytes, and plasma cells. Thus, several investigators have suggested that the Ia antigen is a normal differentiation marker on hematopoietic cells. We have investigated the distribution of Ia antigens on cells of the eosinophil series. The eosinophil precursor cell (CFU-EO) is distinct from the granulocyte-monocyte colony-forming cell (CFU-C). We report evidence indicating that the Ia antigen is present on the human eosinophil colony-forming cell and is absent on mature eosinophils.

MATERIALS AND METHODS

Patients

We studied six normal volunteers, three patients with hypereosinophilic syndrome, and six patients with diseases associated with eosinophilia. This latter group included a patient with Hodgkin's disease in remission, lymphoma in remission, collagen vascular disease, asthma, and two patients with myelogenous leukemia in remission. The three patients with hypereosinophilia were males between the ages of 25 and 40, who had splenomegaly, an eosinophil count greater than 17,000/μl, and no other known cause for the increase in blood eosinophils.

Cells and Antiserum

Heparinized aspirates of bone marrow were obtained from appropriately informed individuals. The mononuclear cells were isolated by centrifugation on Ficoll-Hypaque (900 g, 45 min, 4°C). These cells were washed twice, counted, and used in the cytotoxicity assay for eosinophil colony-forming cells.

Eosinophils from the patients and normal volunteers were purified by a series of isolation steps modified from the technique of Parrillo and Fauci. Heparinized venous blood was centrifuged on Ficoll-Hypaque gradient (900 g, 45 min, 20°C). The cell button was recovered, resuspended in alpha-medium (Flow), and dextran-sedimented (3% dextran in normal saline) at normal gravity. The leukocyte-rich fraction was lysed with ammonium chloride in Tris buffer to remove remaining erythrocytes. This neutrophil-eosinophil mixture was suspended in 3-4 ml alpha-medium containing 20% heat-inactivated fetal calf serum and run through a nylon-wool column (3 g of nylon wool, Leukopak, Fenwal Laboratories, Deerfield, Ill.) packed into a 35-ml syringe. The column was primed with 100 ml alpha-medium containing 5% heat-inactivated fetal calf serum. The cells were washed through the column twice and then incubated in the column for 15 min at 37°C. The neutrophils adhered to the nylon wool, and the eosinophils were eluted with 50–100 ml of alpha-medium at 37°C. Cell viability, as determined by trypan blue exclusion, was greater than 95%. An aliquot of the final cell suspension was used for making cytocentrifuge slides that were stained with Wright-Giemsa or luxol blue.

The expression of the Ia antigen was studied using rabbit anti-human Ia serum. The antiserum was produced by subcutaneous immunization of rabbits with papain digests of membranes from human spleens involved with B-lymphoblastic lymphoma. The antiserum has been studied extensively and has been shown to react with B lymphocytes, early granulocytic precursors, and the majority of acute myelogenous and lymphocytic leukemia cells, but not with T lymphocytes, granulocytes, erythrocytes, or platelets.

Cytotoxicity and Immunofluorescent Assays

The purified eosinophils were tested for Ia antigen expression using the eosin dye exclusion microcytotoxicity assay. The assay was performed as previously described by adding 1 μl undiluted Ia antiserum to 2000 cells in 1 μl alpha-medium in a microtest plate. After 30-min incubation at 22°C, 5 μl absorbed rabbit complement (C) was added, and the cells were incubated for another 60 min. The cells were suspended with eosin dye, fixed, and read for cytotoxicity. Over 300 cells were assayed for each patient or volunteer. Positive cytolysis was arbitrarily defined as equal to or greater than 5% cell kill. The Ia antigen expression was also tested.
by indirect immunofluorescence on two of the normal volunteers, one of the patients with hypereosinophilic syndrome, and two of the patients with diseases associated with eosinophilia. One-million purified eosinophils were incubated for 30 min at room temperature with 50 μl fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories, Springfield, Va.) at a dilution of 1:20. After washing 3 times to remove the excess fluorescein reagent, the cells were examined under ultraviolet light with a Leitz orthoplan microscope. As controls, 1a-antigen-positive B lymphocytes (B-lymphocyte line, Raji) and 1a-antigen-negative T lymphocytes (T-lymphocyte line, Molt-4) were tested by both microcytotoxicity and indirect immunofluorescence.

The CFU-EO cytotoxicity assay was performed with 2 × 10⁶ mononuclear bone marrow cells in 100 μl alpha-medium containing 20% heat-inactivated fetal calf serum with either 100 μl heat-inactivated 1a antiserum or heat-inactivated normal rabbit serum (Fig. 1). The cells were incubated for 30 min at 37°C in an atmosphere of 5% CO₂ in air. Normal rabbit serum (100 μl) was then added to the cells as a source of complement for an additional 60 min. The cells were washed 3 times with phosphate-buffered saline (PBS) and plated in quadruplicate at 3 × 10⁶ viable cells/ml in 0.8% methylcellulose or 0.3% agar. Conditioned medium (100 μl) from a human T-lymphocyte line was added as a source of colony-stimulating factor (CSF). After incubation for 14 days, the number of eosinophil colonies that contained more than 40 cells was determined by staining the entire plate with either Dominici's stain (eosinophil cytoplasmic granules stain dark pink; Fig. 2A), or benzidine dihydrochloride plus potassium cyanide (KCN) stain (eosinophil colonies stain black while granulocytes and monocytes stain light brown because eosinophil peroxidase is not inhibited by cyanide, whereas neutrophil and monocyte peroxidase is inhibited; Fig. 2B). The procedure for the differential staining of eosinophil peroxidase was modified from prior procedures by preincubating the plates with 20 mg/ml KCN in alpha-medium for 1 hr before adding the benzidine dihydrochloride solution.

After the agar culture was stained, the entire plate was submerged in a disposable plastic weigh boat containing 50 ml of distilled water. The agar culture detached and floated on the surface, then was picked up with a 3 × 2 inch microscope slide. A prewetted cellulose acetate membrane was placed over the agar to permit uniform drying. After drying, the cellulose acetate membrane was removed, the slide covered with microscope oil, and the colonies were enumerated. To verify the accuracy of eosinophil colony count using the above staining methods, individual colonies were aspirated from methylcellulose, smeared onto slides, and stained with Wright-Giemsa or luxol blue, and the percent eosinophil colonies counted. Results differed by approximately 20% between the three methods. Mixed colonies of eosinophils with neutrophils or macrophages were not seen.

Absorption studies were performed at a concentration of 10⁸ cells/ml. Antiserum was absorbed with T and B lymphocytes for 1 hr at 37°C, the cells centrifuged at 2000 g for 15 min, and the absorbed antiserum recovered. This procedure was repeated twice. The absorbing T lymphocytes were from a previously described human cell line, and the B lymphocytes were purified from a patient with chronic lymphocytic leukemia.

**RESULTS**

The results of the experiments using the 1a antiserum and bone marrow mononuclear cells from the three groups of patients are given in Table 1. The data indicate that the eosinophil colony-forming cell expressed the 1a antigen. Almost all CFU-EO were inhibited at an antiserum titer of 1:120. Colony inhibition was strictly complement-dependent and complement alone was not inhibitory. The antiserum did not inhibit CFU-EO after absorption with B lymphocytes, but full inhibition was retained after absorption with T lymphocytes. The percentage inhibition did not differ among the normal volunteers and patients with the hypereosinophilic syndrome and diseases with increased peripheral blood eosinophils.

The eosinophil purification data are shown in Table 2. The normal volunteers and patients with hypereosinophilic syndrome and other diseases with eosinophilia had approximately 2%, 73% and 18% eosin-
Evidence suggests that the Ia antigen may represent an early differentiation marker on hematopoietic cells. The Ia antigen is expressed on both normal and leukemic null and B lymphocytes and a subpopulation of activated T lymphocytes, but is not present on most mature plasma cells.\textsuperscript{1,3,7,25,26} It is found on the early erythroid precursors, but not on orthochromic normoblasts and more mature erythrocytes.\textsuperscript{8} The Ia antigen is expressed on granulocyte-monocyte colony-forming cells and normal and leukemic myeloblasts, but is absent or decreased on promyelocytes and is not pres-
ent on more mature granulocytes. In contrast, the Ia antigen is found on the human macrophage, which is a mature progeny of the CFU-C. The antigen is expressed on the megakaryocyte and its colony-forming cell, but is not detected on platelets. In contrast, the data demonstrate that the Ia antigen is a differentia-

tion marker on human eosinophils. A summary of Ia expression on human hematopoietic cells is presented in Fig. 3.

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