basic protein, eosinophil peroxidase, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil lyso- phospholipase.

**Eosinophil-associated Proteins**

**Eosinophil Granule Major Basic Protein**

In 1973 we purified crystalloid-containing granules from guinea pig eosinophils and found that these granules were readily soluble in dilute acid. Analyses of granule extracts by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed a prominent band with a molecular weight of 11,000. When we analyzed the gels by densitometry, we found that this strong band accounted for over half of the protein in the gel. Because this protein was basic, we called it the eosinophil granule major basic protein (MBP).

Amino acid analysis showed 13 residues (13 percent) of arginine in keeping with the basicity of MBP. MBP contains 6 moles of half-cystine and 2 reactive sulphydryl groups, which interact to form disulfide-linked insoluble aggregates. Because of this tendency to polymerize and precipitate, we used reduced and alkylated MBP in many experiments.

Proteins analogous to MBP were also isolated from rat and human eosinophil granules, and they had properties similar to guinea pig MBP, including a high content of arginine.

We used 2 methods to investigate the location of MBP within the granule. The first used immunoperoxidase electron microscopy and showed MBP within the crystalloid core. The second used cell fractionation techniques to isolate the crystalloids. The crystalloids were dissolved in dilute acid and this solution showed: (1) that by SDS-PAGE a single band with 11,000 molecular weight was present and (2) that the protein content as measured by biuret assay could be accounted for by the quantity of MBP measured by radioimmunoassay (RIA). Thus, MBP exists as a crystal which forms the core of the eosinophil granule.

**Eosinophil Peroxidase**

In eosinophils, peroxidase activity is localized to the matrix of the granule, is released into the phagocytic vacuole

---

**Eosinophils and Bronchial Inflammation**

G. J. Gleich, M.D.; D. A. Loegering, B.S.; and C. R. Adolphson, M.S.

**Morphology**

In 1879 Paul Ehrlich stained peripheral blood cells with coal tar dyes and reported that certain cells stained intensely with acid dyes such as eosin. Because of the affinity of these cells for eosin, they were named eosinophils. By optical microscopy, prominent characteristics of eosinophils are a bilobed nucleus and a cytoplasm densely packed with granules. There are two types of granules: the large crystalloid-containing granule and the small granule. Figure 1 shows an electron photomicrograph of an eosinophil with abundant crystalloid-containing granules. These granules have a distinctive substructure, being composed of an electron-dense core and an electron radiolucent matrix. Presumably, some of the eosinophil's activities are mediated through the granule constituents and, therefore, they have served as a focus for research. At the present time there are five recognized proteins derived from eosinophil granules: major

---

*From the Departments of Immunology and Medicine, and the Allergic Diseases Research Laboratory, Mayo Clinic and Mayo Foundation and the Mayo Medical School, Rochester, MN. Supported in part by grants AI 07928 and AI 15231 from the National Institute of Allergy and Infectious Diseases and from the Mayo Foundation.

*Reprint requests: Dr. Gleich, Mayo Clinic, Rochester, Minnesota 55905*
following ingestion of zymosan or *Escherichia coli*; and is deposited onto the surface of parasites. Purified eosinophil peroxidase (EPO) exists as a monomer of 75,000 daltons and a dimer of 150,000 daltons as measured by gel filtration. The EPO catalyzes the oxidation of many substances by H$_2$O$_2$, and because eosinophils generate considerable H$_2$O$_2$, the activities of the EPO + halide + H$_2$O$_2$ system in the killing of microorganisms have been explored. These results indicated that many organisms can be killed, including Schistosomula, Toxoplasma, and Trypanosoma. EPO + halide + H$_2$O$_2$ also caused mast cell degranulation and histamine release. Finally, EPO can absorb to tumor cells and such cells are spontaneously lysed by macrophages; this finding points to a synergistic action between the cytoplasmic cationic EPO and H$_2$O$_2$ which is spontaneously released from macrophages in tumor cell destruction.

**Eosinophil-derived Neurotoxin**

A second molecule derived from the eosinophil granule is the eosinophil-derived neurotoxin (EDN). EDN is a powerful neurotoxin which can severely damage myelinated neurons in experimental animals. In 1933, an English investigator, M. H. Gordon, described a new test for Hodgkin's disease. He injected extracts of spleen and lymph nodes from patients with Hodgkin's disease into healthy rabbits. The rabbits became ill with a paralytic disease and associated disequilibrium; this reaction came to be known as the "Gordon phenomenon." Histologically, a hallmark of the Gordon phenomenon is the disappearance of Purkinje's cells from the cerebellum. In addition, the white matter of cerebellum, pons, and spinal cord shows a gross spongiform change; the gray matter remains essentially normal. It was discovered later that the Gordon phenomenon was due to the presence of eosinophils in the spleen and lymph nodes and could also be induced by injecting eosinophil homogenates.

Using the Gordon phenomenon as an assay, Durack et al demonstrated EDN activity in eosinophil extracts and showed that neurotoxic activity eluted at an apparent molecular weight of 15,000. Subsequently, extracts of whole human eosinophils and purified eosinophil granules were sequentially fractionated by gel filtration at acid and alkaline pH. Fractions were analyzed by SDS-PAGE for their ability to produce the Gordon phenomenon by intrathecal injection into rabbits. Fractionation of human eosinophil granule extracts on Sephadex G-50 at pH 4.3 yielded three major peaks. Eosinophil enzymes including peroxidase are included in peak 1, while MBP constitutes the protein in peak 3. Neither peak 1 nor peak 3 protein produced the Gordon phenomenon. Fractions composing peak 2 were concentrated and fractionated on Sephadex G-50 at pH 7.4. This purified material caused the Gordon phenomenon, and by SDS-PAGE the active material gave a single band with a molecular weight of 17,913. In these same studies, purified Charcot-Leyden crystal protein (lyso phospholipase) and MBP did not produce the Gordon phenomenon. Recently, Peterson and Venge described eosinophil protein X (EPX); EPX produces the Gordon phenomenon and has properties similar to EDN, and the 2 may be identical. In these studies, the eosinophil cationic protein (ECP) was about 100 times more active than EPX in producing the Gordon phenomenon.

**Eosinophil Cationic Protein**

A third molecule of interest from the eosinophil granule is the eosinophil cationic protein (ECP) first described in 1974 by Olsson and Venge. ECP is probably localized in the matrix of the crystallloid-containing granule, constitutes about 30 percent of the granule protein; has an isoelectric point greater than 11; contains 18 different amino acids including 21 residues of arginine, 10 half-cystine residues, and a high content of aspartic and glutamic acid; is not bactericidal or erytolytic; binds to heparin and neutralizes its anticoagulant activity; augments factor XII-dependent pathways; and damages Schistosomula of *S mansoni*. We recently compared ECP with EDN and MBP and we were able to show the distinctive nature of these proteins by electrophoresis on SDS-PAGE, by specific double antibody RIA for MBP and ECP, and by gel filtration of acid solubilized eosinophil granules.

**Eosinophil Lyso phospholipase (Charcot-Leyden Crystal Protein)**

The Charcot-Leyden crystal (CLC) was initially described in 1853 in a patient with leukemia and later in 1872 in the sputa of patients with asthma. Since then the appearance in tissues and body fluids of these hexagonal bipyramidal crystals has been a hallmark of the eosinophil. In 1980 Weller et al, while working with enzymes preferentially present in the eosinophil, found that crystals of CLC formed when purified eosinophil lyso phospholipase activity was concentrated. Eosinophil lyso phospholipase catalyzes inactivation of lyso phospholipids. Recently, we found lyso phospholipase antigenic activity in both eosinophils and basophils. Purified CLC dissolves in acid or detergent and shows a single band that stains for protein after SDS-PAGE. The molecular weight of CLC is 12,980 ± 233 (X ± SD; n = 5); amino acid analyses found 6 residues of arginine, 18 to 19 of glutamic acid, and 12 of valine. Several lines of evidence suggest that lyso phospholipase (CLC protein) is localized in the plasma membrane of the eosinophil. For example, granule fractions showed no lyso phospholipase activity, whereas activity was found in fractions containing magnesium-dependent ATP activity, a membrane marker.

**Eosinophils and Asthma**

In studies to define the biologic activity of MBP, we tested whether schistosomes of *S mansoni* were damaged by MBP. MBP-treated schistosomes were damaged as shown by destruction of the cuticle, by formation of blebs on the membrane and by eventual dissolution of the organism into amorphous debris. Subsequently, it was shown that the incubation of eosinophils with schistosomes in the presence of antibody resulted in the deposition of MBP on the surface of the parasite and increased concentrations of MBP could be detected in culture supernatants. These studies showed that MBP was toxic to schistosomes and that MBP was deposited onto the organisms and released into the culture supernatant. During the studies described above, MBP also killed murine tumor cells, and in subsequent experiments we
tested the toxicity of MBP on cells from organs which are infiltrated by eosinophils in disease states. We found that MBP damaged intestinal, splenic, cutaneous, and peripheral blood mononuclear cells in a dose-related manner.36 We next observed that MBP damaged guinea pig tracheal rings in tissue culture,77 and it also damaged human bronchial epithelium in tissue culture.67 In these tissue culture experiments, there was extensive damage to the epithelium, with detachment of ciliated and brush cells and destruction of individual cells, leaving only basal cells. Exfoliated cells were severely damaged, with lysis of the cellular membrane and liberation of cell contents. Cilia were stripped from cells. The normal microtubular structure of the axonemes was lost. The lamina propria appeared edematous, with separation of collagen fibrils. These effects of MBP on bronchial epithelium were remarkably similar to the histopathologic changes seen in human bronchial asthma. Specifically, excessive shedding and desquamation of the bronchial epithelium, down to the level of the lamina propria, are reported as constant findings in bronchial asthma.68 The superficial columnar cells undergo detachment, leaving behind a layer of basal cells, and from these regeneration of the mucosa takes place. Finally, mucociliary clearance is impaired in asthma.69

We recently collaborated with O'Donnell and Thomas38 to investigate the role of human MBP in triggering noncytolytic histamine release from human leukocytes. We tested both the basophil-containing mononuclear cell fraction isolated by Ficoll-Hypaque density gradient centrifugation of peripheral blood and suspensions of 92 to 96 percent human basophils which had been purified by flow microfluorometry; MBP caused histamine release from the cells in both preparations. The MBP-induced histamine release was not a toxic effect because it was calcium-, energy-, and temperature-dependent. Therefore, human MBP directly induces noncytolytic histamine release from human basophils.

With the above information on MBP, we addressed the question of the relationship of eosinophil MBP to human disease. Because of the striking similarity between the damage caused by MBP in vitro to respiratory tissue and respiratory tissues damaged during bronchial asthma, we tested the hypothesis that eosinophils mediated bronchial injury. First, we analyzed sputa from 100 consecutive patients with various respiratory diseases for MBP by RIA.80 In 73 patients the MBP levels were below the sensitivity of the assay, and only 1 had bronchial asthma; in the remaining 27 patients with measurable MBP, 12 had bronchial asthma. Of the 13 patients with a sputum level >0.1 μg/ml, 11 had bronchial asthma. We also studied sputum specimens from 15 patients hospitalized for asthma. The peak MBP levels ranged from 0.3 μg/ml to 92 μg/ml, with a geometric mean of 7.1 μg/ml, in our tissue culture studies, 10 μg/ml caused epithelial damage in vitro.87 These results indicated that an elevated sputum MBP was a good marker for bronchial asthma, and that quite high concentrations of MBP were present in the sputa of some patients with asthma. Certain sputa processed within a few minutes after expectoration contained high MBP levels, suggesting that the elevation of MBP in the sputa was not merely a consequence of in vitro cell death and liberation of granule contents.

Second, we examined lung tissues obtained at autopsy from patients who died of asthma to look for intracellular and extracellular MBP by immunofluorescence.80 The results showed bright fluorescence of eosinophils in the lung tissues and extracellular staining of MBP along the bronchiole lining in association with areas of damage to bronchial epithelium as well as in mucous plugs, these plugs partially obstructed the lumen in the small bronchioles. The detection of extracellular MBP suggests that the eosinophil degranulates into the bronchial lumen in asthma.

Finally, the recent demonstration that eosinophils preferentially produce leukotriene C, as compared to neutrophils,81 suggests that they may induce bronchospasm directly. Eosinophils could induce mediator secretion from lung mast cells in asthma either through the action of EPO + H2O2 + halide or through MBP. As noted above, MBP activates human basophils and rat mast cells to release histamine in an energy-, temperature-, and calcium-dependent manner.82 More recent observations indicate that MBP stimulates wheal-and-flare skin reactions in a dose-related manner83 and that several eosinophil granule proteins, including EPO, EDN, and ECP, are also active in triggering wheal-and-flare reactions.84 Taken together, these findings point to the possibility (Fig 2)85 that the eosinophil acts as an important effector cell in chronic asthma.

REFERENCES

Mast Cell Mediators in the Blood of Patients with Asthma*

Stephen I. Wasserman, M.D.

The identification of human mast cell-dependent disease is usually made on historic grounds together with appropriate physical signs and symptoms. In asthma, these findings include reversibility of signs and symptoms, the characteristic triggers of airways dysfunction, and the cardinal signs of airway obstruction, including wheeze and cough. These findings are supported by objective measurement of pulmonary function.

Accumulating evidence suggests that in addition to signs, symptoms, and pulmonary functional abnormalities which are reversed after β-adrenergic agonist inhalation, it may be possible to identify in the blood markers suggestive of mast cell-mediated bronchoconstriction. This review will focus upon those mediators identified in human diseases with

*From the Division of Rheumatology/Allergy, University of California Medical Center, San Diego.

Reprint requests: Dr. Wasserman, UCSD Medical Center, 225 West Dickinson Street, San Diego 92037