Comparative Properties of the Charcot-Leyden Crystal Protein and the Major Basic Protein from Human Eosinophils

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ABSTRACT Guinea pig eosinophil granules contain a protein, the major basic protein (MBP), which accounts for more than half of the total granule protein, has a high content of arginine, and displays a remarkable tendency to form disulfide-linked aggregates. In this study we have purified a similar protein from human eosinophil granules and have compared the human MBP to the protein comprising the Charcot-Leyden crystal (CLC). Eosinophils from patients with various diseases were purified and disrupted, and the granule fraction was obtained. Examination of the granule fraction by transmission electron microscopy showed numerous typical eosinophil granules. Analyses of granule lysates by gel filtration and by polyacrylamide gel electrophoresis revealed the presence of peroxidase and MBP with properties similar to that previously found in guinea pig eosinophil granules. The human MBP had a molecular weight of 9,200, contained less than 1% carbohydrate, was rich in arginine, and readily formed disulfide-bonded aggregates.

CLC were prepared from eosinophil-rich cell suspensions by homogenization in hypotonic saline. The supernates following centrifugation of cell debris spontaneously formed CLC. Analysis of CLC revealed the presence of a protein with a molecular weight of 13,000 containing 1.2% carbohydrate. The protein displayed a remarkable tendency to aggregate even in the presence of 0.2 M acetic acid.

Human MBP and CLC protein differed in their molecular weights, carbohydrate compositions, and amino acid analyses. Mixtures of the MBP and the CLC protein yielded two bands in polyacrylamide gel electrophoresis. Neither eosinophil protein increased vascular permeability in the guinea pig skin or contracted the guinea pig ileum. The results indicate that the human MBP and the CLC are distinct substances with properties such that one cannot be derived from the other.

INTRODUCTION

In 1853 Charcot and Robin (1) noted the presence of a distinctive crystalline material in the spleen of a patient with leukemia, and subsequently, Leyden described similar crystals in the sputum of individuals with bronchial asthma (2). These crystals, termed "Charcot-Leyden crystals" (CLC),[1] have been found in a variety of conditions associated with eosinophilia (3). However, little is known about the properties of the CLC. Buddecke and his associates detected 14 amino acids in CLC (4) and Hornung found that CLC were composed of a polypeptide of low molecular weight containing tyrosine (5). Archer and Blackwood obtained CLC from both human eosinophils and basophils and concluded that CLC were composed of protein and that they formed from cytoplasm and not from granules or nuclei (6).

In prior studies we purified granules from guinea pig eosinophils and identified a basic protein which accounted for the bulk of the granule protein. This material, termed the "major basic protein" (MBP), had a molecular weight of 11,000, contained 13% arginine, and displayed a striking tendency to form disulfide-linked aggregates (7, 8). The MBP did not mediate increases in vascular permeability, nor did it contract the guinea pig ileum.

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[1] *Abbreviations used in this paper:* CLC, Charcot-Leyden crystals; DTT, dithiothreitol; MBP, major basic protein; SDS, sodium lauryl sulfate.

In the present study we have purified eosinophils from the blood of humans with various diseases and have isolated the granules from the purified cells. Analyses of granule proteins indicate that they also contain a MBP similar to that found in the guinea pig. CLC were prepared from the eosinophils of the same patients and their properties compared to those of the MBP. The results indicate that these are different substances with characteristics such that one cannot be derived from the other.

METHODS

Reagents. Sucrose, pyronin-Y, and hydrogen peroxide were obtained from the Fisher Scientific Company, Pittsburgh, Pa. Dithiothreitol (DTT), iodoacetamide, histamine, *o*-dianisidine, and bradykinin were purchased from Sigma Chemical Company, St. Louis, Mo. *p*-Toluene sulfonic acid and formic acid were obtained from Calbiochem, La Jolla, Calif. Bio-Gel Agarose A, 0.5 M, was purchased from Bio-Rad Laboratories, Richmond, Calif. Dextran 500,000 was purchased from Pharmacia Fine Chemicals, Piscataway, N. J. Sodium lauryl sulfate (SDS) was obtained from Schwarz-Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.

Patients. Eosinophils were obtained from five patients who had marked peripheral blood eosinophilia. Patient 1 had an eosinophilic pneumonia which subsequently responded to treatment with prednisone. She presently is well, but she requires intermittent treatment with prednisone. At the time of study, she had a leukocyte count of 16,300 per mm^3 with 45% eosinophils. Patient 2 had a chronic dermatitis with a leukocyte count of 36,300 per mm^3 with 67% eosinophils. She had prominent lymphadenopathy, but repeated biopsies did not disclose evidence of lymphoma. Patient 3 had a syndrome characterized by marked peripheral blood eosinophils and episodes of angioedema involving the extremities and eyelids. He was admitted to the Clinical Study Unit and the administration of prednisone temporarily stopped. The patient experienced a dramatic flare of his disease with a weight gain of 30 lb and eosinophilia as high as 90,000 cells per $mm.^3$ Treatment with corticosteroids resulted in a diuresis of 30 lb and a complete remission. Subsequently, the patient had required prednisone up to 30 mg per day to control his symptomatology. Leukocytes were obtained twice from this patient, and on these occasions his total leukocyte counts were 33,000 and 46,000 cells per mm^3 of which 53% and 75%, respectively, were eosinophils. Patient 4 probably has eosinophilic leukemia with cardiac involvement and marked peripheral blood eosinophilia. He had been treated with hydroxyurea as well as with prednisone and continues to have cardiac murmurs and borderline congestive heart failure. Patient 5 has an apparently unique syndrome characterized by episodic edema and pretibial nodules associated with abnormalities of immunoglobulins and complement. The detailed findings on this patient will be presented in a separate report. Over the past 8 yr her total leukocyte count has averaged about 13,000 cells per mm^3 with 50–60% eosinophils.

Purification of eosinophils. In preliminary experiments we investigated the conditions necessary for purification of human eosinophils and found that the use of sodium diatrizoate at a density of 1.142 represented the best compromise between the needs for a substantial yield and high purity (9, 10). Eosinophils were obtained from the first four patients with the IBM-NCI cell separator (11). Up to 10 liters of blood were processed in this device and the leukocyte-rich fraction obtained. The leukocytes were separated from erythrocytes by addition of 1 vol of 4.5% dextran in 0.9% sodium chloride to 3 vol of the leukocyte-rich fraction and sedimentation at 1 *g* in 50-ml plastic syringes. 8 ml of the leukocyte-rich plasma supernate was layered with care over 8 ml of sodium diatrizoate ($d = 1.142$) in a 30-ml polycarbonate tube and centrifuged (Model K, Damon/IEC Div., Damon Corp., Needham Heights, Mass.) for 40 min at 600 *g*. Care was taken during acceleration to avoid abrupt increases in the application of the centrifugal force. After centrifugation the clear supernate was removed from the top layer of the tube, and the cells at the interface between plasma and diatrizoate were collected (interface layer) with a Pasteur pipette. The suspension of cells in diatrizoate (diatrizoate layer) was aspirated, after which the cells in the pellet were harvested. In some experiments the purity of the eosinophils from the pellet was quite high, but in no case was it above 90%. The purities of eosinophils in the sodium diatrizoate and interface layers were correspondingly less. In some experiments cells from the pellets were suspended in 0.9% NaCl and allowed to settle at room temperature. After 15–30 min these cells separated into two layers, and the bottom layer was often enriched in eosinophils with final purities of 90–96%. In all of the experiments dealing with granules we used cells from the pellet as the starting preparations. The cells contaminating these preparations were neutrophils and occasional mononuclear cells.

Preparation of eosinophil granules. We used essentially the same procedure described previously for preparation of granules from guinea pig eosinophils (7), but we found that the addition of sodium heparin as suggested by Chodirker and his associates was a critical modification (12). Suspensions of cells in 0.34 M sucrose containing 250 U of heparin per ml were repeatedly pipetted, and during the course of this procedure the suspensions of cells became exceedingly viscous so that addition of extra volumes of the disrupting solution was necessary. After repeated mixing the suspensions were centrifuged for 10 min at 400 *g* at 4°C to remove unbroken cells. The opalescent supernates were transferred to another tube and centrifuged at 25,000 *g* for 20 min at 4°C. The pellet was solubilized in 0.02 M, pH 4.3 acetate buffer, in 0.01 M HCl, or in 0.15% NaCl and analyzed by electrophoresis on polyacrylamide gels or by gel filtration on columns of Sephadex G-50.

For example, 158 ml of leukocyte-rich cell suspension was obtained from patient 3 by the IBM-NCI cell separator and contained a total of 4.27×10^{10} leukocytes, of which 2.28×10^{10}, or 53%, were eosinophils. After addition of 53 ml of the 4.5% dextran solution and sedimentation at 1 *g* the plasma layers were pooled, yielding a volume of 133 ml containing a total of 3.90×10^{10} total leukocytes, of which 2.26×10^{10}, or 59%, were eosinophils. 8-ml samples of the leukocyte-rich plasma were layered over 8-ml volumes of sodium diatrizoate ($d = 1.142$) in 16 tubes. After centrifugation the pellets were resuspended and pooled, and the cell suspension, 90 ml, contained a total of 6.7×10^9 total leukocytes, of which 5.5×10^9, or 86%, were eosinophils. For preparation of granules the purified eosinophils were washed in 0.34 M sucrose, and 1.8×10^9 cells were suspended in each of three tubes in a volume of 20 ml of 0.34 M sucrose containing 250 U of heparin per ml and repeatedly pipetted. The suspended cells became extremely viscous during this step. Therefore, the individual samples were pooled, and 300 ml of 0.34 M sucrose containing 250 U of heparin per ml

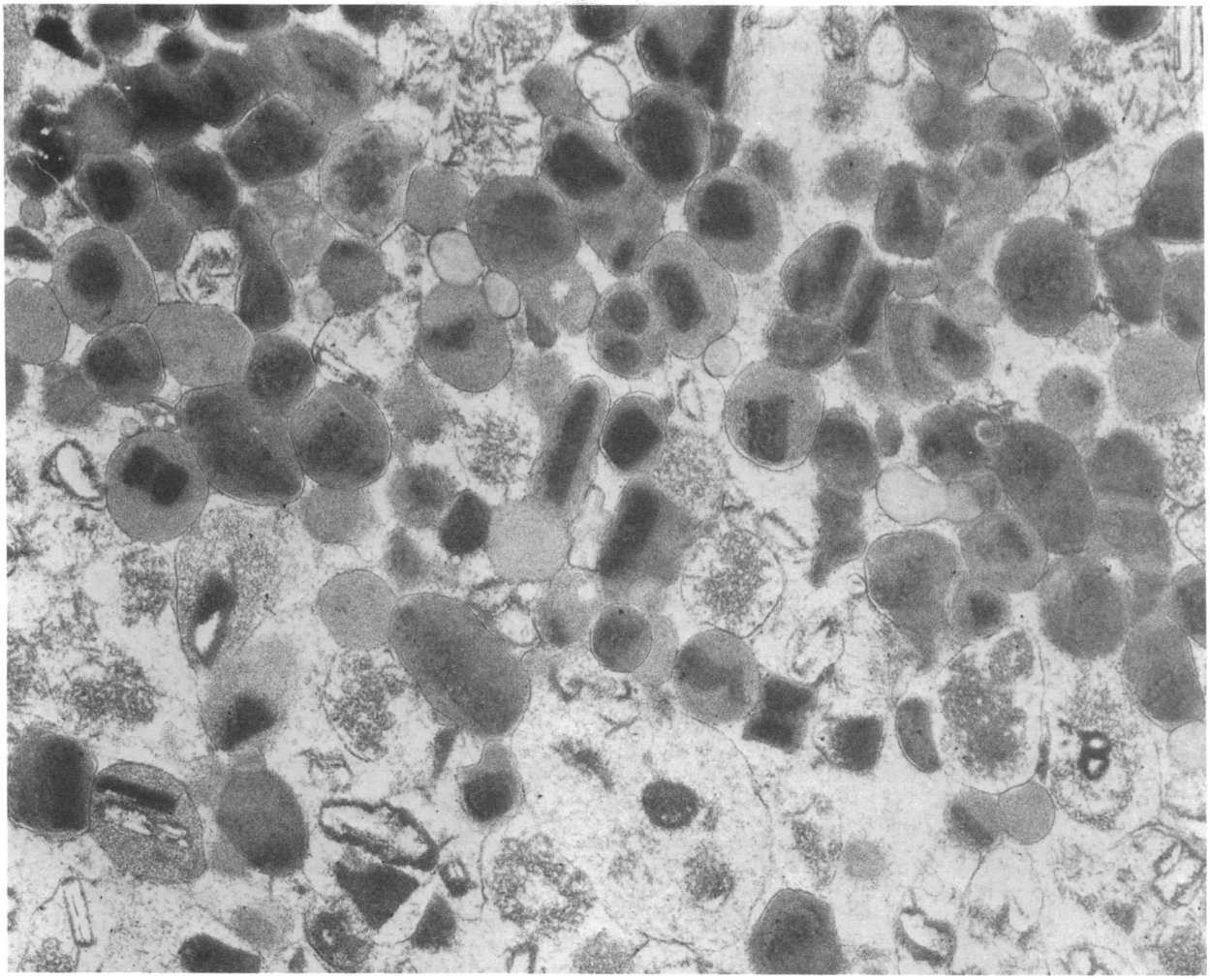

FIGURE 1 Transmission electron photomicrographs of purified human eosinophil granules from patient 1. Granules were fixed with glutaraldehyde and stained with uranyl acetate and lead citrate. Numerous membrane-bounded typical granules are present with a core and matrix while other granules are in various stages of degeneration. × 18,500.

was added to obtain a product which could be forced through a syringe. Finally, an additional 200 ml of 0.34 M sucrose without heparin was added, and the material was transferred to 40-ml polycarbonate tubes and centrifuged at 800 *g* for 10 min to remove unbroken cells. The eosinophil granules were recovered by centrifugation at 25,000 *g* for 10 min with a Beckman J-21 centrifuge with a JA-20 rotor (Beckman Instruments, Inc., Palo Alto, Calif.).

Preparation of CLC. In preliminary experiments we investigated a variety of methods to prepare CLC, but we found that the most useful procedure was that employed by Archer and Blackwood (6). Cells from the interface and diatrizoate layers obtained after sedimentation in sodium diatrizoate were subjected to hypotonic shock to remove erythrocytes by suspension in a solution of 0.15% NaCl. The cells were washed in 0.9% NaCl and were suspended in 0.15% sodium chloride and repeatedly homogenized with a motor-driven (Talboys Engineering Corp., Emerson, N. J.) Potter-Elvehjem Teflon tissue grinder (Kontes Glass Company, Vineland, N. J.; size 22). Usually, 20–30 passes were required until a smooth suspension was obtained. The suspensions were centrifuged for 5 min at 5,000 *g* to remove the bulk of the cellular debris and again for 30 min at 10,000 *g* to yield clear supernates. These supernates were kept at 4°C overnight and examined for the presence of CLC by microscopy the following morning. On several occasions we concentrated the supernatant solutions by pervaporation in order to prepare additional quantities of CLC or to obtain CLC from preparations which did not crystallize during the initial incubation at 4°C. The CLC suspensions were repeatedly washed with 0.15 M sodium chloride and examined by phase microscopy to determine their homogeneity. Preparations judged of sufficient purity were analyzed for their physiochemical and biological properties.

Polyacrylamide gel electrophoresis. Solubilized eosinophil granules and CLC were analyzed by electrophoresis in 8% polyacrylamide gels containing 1% SDS by the proce-

dure described by Fairbanks et al. (13). Gels were stained as described by Fairbanks et al. (13).

Peroxidase assay. Peroxidase activity was measured by the rate of decomposition of hydrogen peroxide with *o*-dianisidine as a hydrogen donor as described previously (7).

Electron microscopy. Eosinophil granules were examined by transmission electron microscopy as described previously (7).

Physiochemical analyses of the eosinophil proteins. The eosinophil proteins were analyzed for their protein content by the biuret procedure (14) and for their content of carbohydrate by the phenol-sulfuric acid procedure (15). Amino acid analysis was performed as described by Spackman and his associates (16) with a Beckman model 119 automatic amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Samples were hydrolyzed for 24, 48, and 72 h in 6 M HCl at 110°C. For the determination of cysteine the proteins were oxidized with performic acid as described by Moore (17) and Hirs (18) and then hydrolyzed as described above. For determination of tryptophan the proteins were hydrolyzed with *p*-toluenesulfonic acid as described by Liu and Chang (19). The apparent molecular weights of the eosinophil proteins were determined by gel filtration of the reduced carboxymethylated protein on a calibrated 10% agarose column equilibrated with 6 M guanidinium chloride (20, 21). Briefly, the agarose column is calibrated with a series of polypeptides of known amino acid sequence in terms of N, the number of amino acid residues in the protein. The value of N determined experimentally by the elution of the sample when combined with $\bar{M}_0$, the mean residue weight determined from composition studies, provides a measure of M, the molecular weight of the protein, $M = N \times \bar{M}_0$. The determination of M by this technique is accurate to ±5% and depends upon the assumption that the protein behaves as a linear random coil in 6 M guanidinium chloride.

Biological analyses of the eosinophil proteins. The ability of the eosinophil proteins to alter vascular permeability was tested in 400-g guinea pigs as described by Miles and Miles (22). Guinea pigs were injected intravenously with 0.5 ml of 1% Evans blue dye in 0.9% saline after the skin of the back had been shaved. Various materials were injected and the size and intensity of bluing was measured 15 min after intracutaneous injection of the test materials.

The ability of the eosinophil proteins to contract the isolated guinea pig ileum by themselves or in the presence of histamine or bradykinin was tested by using a 5-ml bath and recording device from Adaps, Inc., Dedham, Mass., as described previously (8).

RESULTS

Eosinophil granules. In all experiments in which we used highly purified preparations of eosinophils, distinct pellets were found at the completion of the granule isolation procedure. Examination of eosinophil granules prepared in this manner by transmission electron microscopy revealed structures with the typical features of eosinophil granules, but the purity was somewhat less than that seen in experiments with the guinea pig eosinophils. A photomicrograph of purified human eosinophil granules is shown in Fig. 1.

Eosinophil granules were solubilized in 0.01 N HCl and stored at −70°C, after which the clear slightly greenish solutions were analyzed by SDS polyacrylamide gel electrophoresis. The electrophoretic pattern shown in Fig. 2 is remarkably similar to that seen with solubilized guinea pig eosinophil granules (7) with a

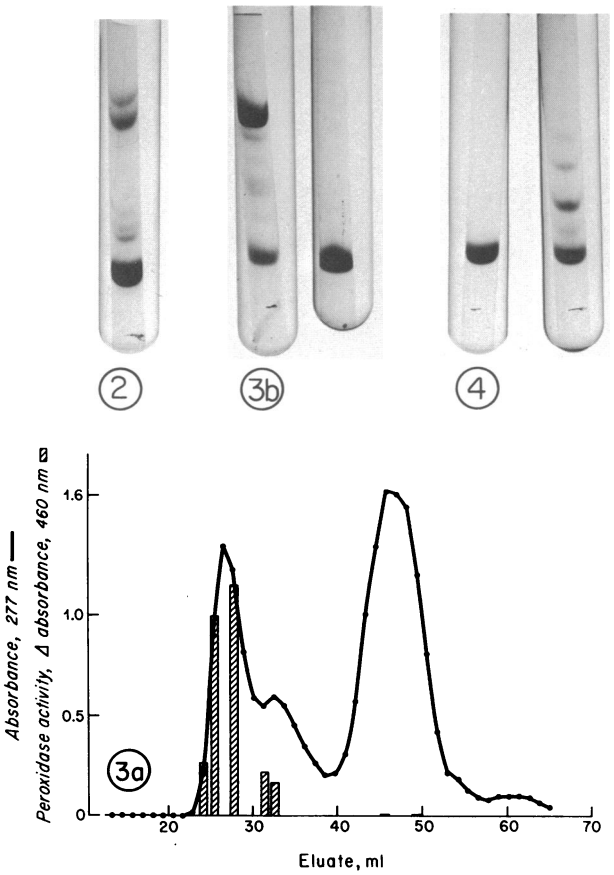

FIGURE 2 Analysis of human eosinophil granules from patient 5 by SDS polyacrylamide gel electrophoresis. In this and in all other polyacrylamide electrophoreses the anode is at the bottom. The stab mark in the gel indicates the distance traveled by the pyronin-Y marker. The granule proteins in this experiment were not treated with DTT.

FIGURE 3 Analysis of human eosinophil granule proteins from patient 3 by gel filtration. (*a*) Granules prepared from purified eosinophils were disrupted by freeze-thawing in 0.15% NaCl and fractionated on a 1.2 × 50-cm Sephadex G-50 column equilibrated with 0.025 M, pH 6.6 Na_2HPO_4-NaH_2PO_4. (*b*) Fractions with the greatest absorbance from the first peak eluting at 23–30 ml and the second peak eluting at 40–50 ml were analyzed by electrophoresis on SDS polyacrylamide gels. The peak 1 fraction is on the left and the peak 2 fraction is on the right.

FIGURE 4 Aggregation of purified human MBP. MBP purified from eosinophil granules of patient 1 by fractionation on Sephadex G-50 was stored at −70°C under nitrogen. The sample on the right was analyzed by SDS polyacrylamide gel electrophoresis without addition of DTT whereas the sample on the left was reduced with DTT. The faint band immediately above the MBP on the right has a calculated molecular weight of 14,000 while the remaining bands have molecular weights which are multiples of 9,200.

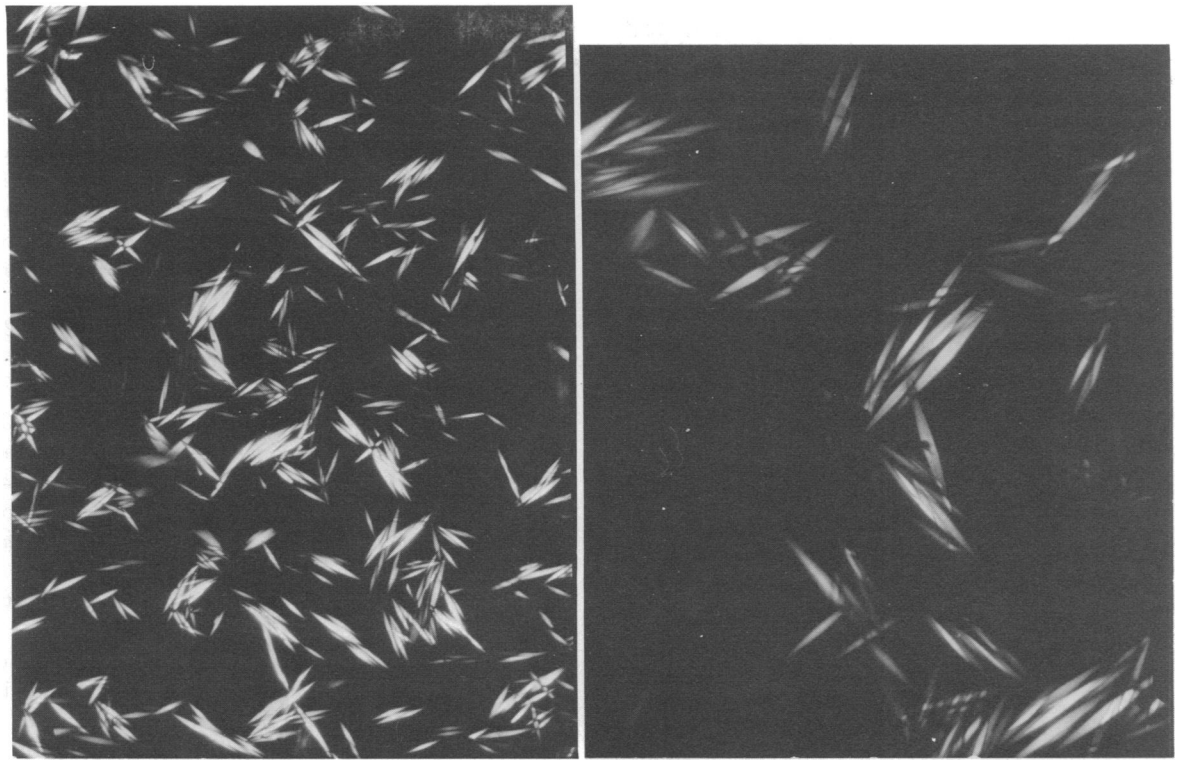

FIGURE 5 CLC from patient 2. The photomicrograph on the left (160 ×) shows the purity of the CLC while that on the right (400 ×) shows the typical morphology of the CLC.

major protein band migrating just above the pyronin-Y marker and a series of bands migrating more slowly in the gel. Similar results were found when purified granules from three other patients were analyzed by SDS polyacrylamide gel electrophoresis. Fractionation of the granule solutions on Sephadex G-50 yielded the pattern shown in Fig. 3. As in the prior studies of guinea pig eosinophil granules, peroxidase activity emerged in the first peak eluting from 23–30 ml whereas the second peak eluting from 40–50 ml was devoid of this activity. Analysis of fractions from the first peak by electrophoresis on SDS polyacrylamide gels revealed one densely staining band in the upper one-third of the gel as well as several other bands while analyses of the fractions from the second peak revealed a single band. After storage at 20°C the substance in the second peak displayed a marked propensity to aggregate as shown in Fig. 4. These aggregates had molecular weights consistent with the formation of a series of polymers from a 9,300-dalton monomer. If the aggregated protein was reduced with DTT before electrophoresis, a single band was found. Because of the similarity of this material to the protein previously isolated from guinea pig eosinophil granules, we shall refer to the material in the second peak as the human "major basic protein."

Preparation of CLC. In preliminary experiments we investigated a variety of conditions to produce CLC, including incubation at various pH's and with various detergents. We found that pH values near neutrality, freedom from serum proteins, and the use of detergents such as Brij 35 (Atlas Chemical Industries Inc., Wil-

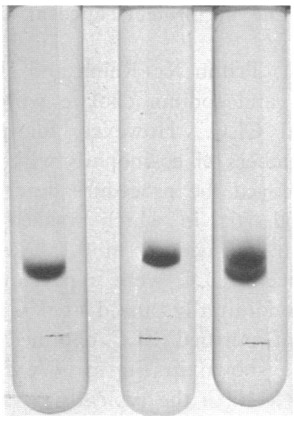

FIGURE 6 Analysis of human MBP and CLC from patient 3 by electrophoresis on SDS polyacrylamide gels. MBP was analyzed on the gel at the left, CLC in the middle gel, and the mixture of MBP and CLC on the right.

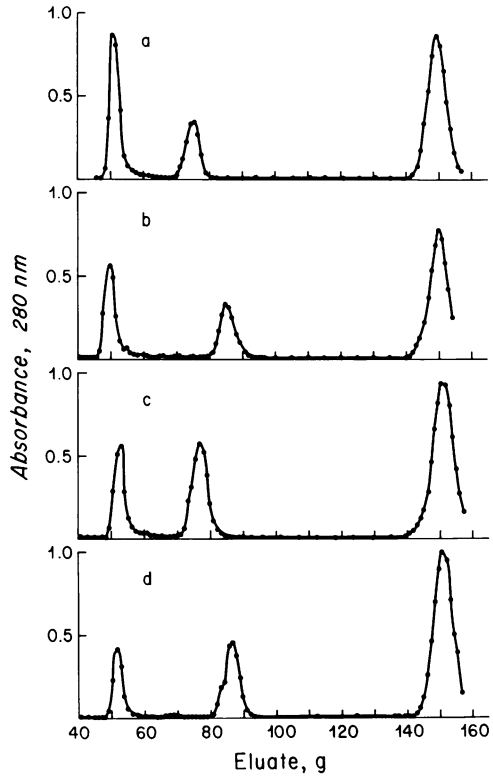

FIGURE 7 Comparisons of MBP and CLC by gel filtration. Samples of reduced and carboxymethylated MBP and CLC were analyzed by gel filtration on a calibrated 10% agarose column equilibrated with 6 M guanidinium chloride. The lyophilized proteins were dissolved in 6 M, pH 8.6 guanidinium chloride. The first peak eluting from the column at the void volume, approximately 50 g, is blue dextran; the last peak eluting from the column at the void volume plus internal volume, approximately 150 g, is DNP-alanine. In (*a*) and (*c*) CLC from patients 2 and 3, respectively, were analyzed, and the protein eluted at about 76 g. In (*b*) and (*d*) MBP from patients 1 and 3, respectively, were analyzed and the protein eluted at about 86 g.

mington, Del.), Triton X (Rohm and Haas Co., Philadelphia, Pa.), and sodium cholate were useful in obtaining typical CLC. However, for producing CLC from large numbers of eosinophils without using detergents we employed the procedure described by Archer and Blackwood (6) in which washed eosinophil-rich preparations are suspended in 0.15% sodium chloride and homogenized. CLC were repeatedly washed with 0.9% NaCl and initially stored at 4°C in 0.9% NaCl. An example of purified CLC obtained from the cells of patient 2 is shown in Fig. 5. We found that after storage in 0.9% NaCl the typical dipyramidal structure of the CLC was altered with blunting of the tips and a loss of structure along the edges. Therefore, in later experiments CLC were washed repeatedly with distilled water and lyophilized. Before we carried out physiochemical studies we analyzed the CLC by SDS polyacrylamide gel electrophoresis to determine their homogeneity. Preparations free of extraneous debris gave a single band. We made a few attempts to purify the CLC protein from higher molecular weight contaminating proteins by gel filtration, but we found that the CLC eluted from columns of Sephadex G-50 equilibrated with 0.025 M, pH 4.3 acetate buffer over a broad range from the void volume to elution volumes characteristic of cytochrome *c* (12,400 daltons). Similar results were found with columns equilibrated with 0.2 M acetic acid and indicate a remarkable tendency of the protein to form aggregates.

Physiochemical analyses of eosinophil proteins. The CLC and the MBP were analyzed by several methods to compare their physiochemical properties. First, analysis by SDS polyacrylamide gel electrophoresis as shown in Fig. 6 revealed that each of the proteins yielded a single band with roughly similar mobilities. When the two proteins were mixed, two bands formed, indicating that they were separate entities. This experiment was repeated on three occasions with MBP and CLC from different patients as well as from the same patient, and identical results were obtained. By SDS polyacrylamide gel electrophoresis the MBP had a molecular weight of 9,300 whereas the CLC had a molecular weight of approximately 13,000. Second, analysis of the reduced and alkylated proteins on the 10% agarose column equilibrated with 6 M guanidinium chloride revealed a difference in their elution volumes as shown in Fig. 7. The molecular weights for the proteins are shown in Table I, and the values for the MBP from the guinea pig are listed for comparison. All of the analyses for guinea pig and human eosinophil proteins were performed on the same 10% agarose column. Third, comparison of the amino acid composition of the proteins revealed marked differences as shown in Table II. The MBP contained a total of 10 or 11 residues of arginine while only 6 arginine residues were present in the CLC. Six half-cystine residues were present in the MBP while only two half-cystine residues were found in the CLC protein. Additional differences between the proteins in amino acid composition were their contents of serine, glutamic acid, valine, methionine, leucine, and lysine. The carbohydrate content of the eosinophil proteins was 0.8% for the MBP and 1.2% for the CLC. The extinction coefficient for the MBP was $E_{1\,cm}^{1\%} = 26.3$ and for the CLC, $E_{1\,cm}^{1\%} = 12.0$. These values were obtained by biuret analyses with human serum albumin as a standard.

Analyses of biological properties of eosinophil proteins. The ability of the eosinophil proteins to induce an increase in vascular permeability in the guinea pig was tested in three animals. In all cases injection of 1 μg of histamine resulted in an area of bluing approxi-

TABLE I

Molecular Weights of the Eosinophil Proteins

	Guinea pig MBP*	Human MBP		CLC		
		Pt 1	Pt 3	Pt 1	Pt 2	Pt 3
SDS polyacrylamide	11,102	9,257	9,490	12,220	13,675	12,637
Gel filtration in 6 M guanidinium HCl	10,800	9,184	9,251	—	12,950	13,369

* From reference 8.

mately 12 × 12 mm whereas the saline controls gave trace bluing no greater than 2 mm in diameter. Injection of CLC dissolved in 0.025 M, pH 4.5 acetate buffer gave reactions not distinguishable from the saline controls whereas injection of human MBP up to 10 μg yielded reactions only slightly greater than control averaging 3 × 3 mm in diameter. Exposure of the human MBP to oxygen in order to induce polymerization did not result in any increase in its ability to induce vascular permeability. The ability of the eosinophil proteins to contract the guinea pig ileum was tested, and neither of them contracted the guinea pig ileum nor did they antagonize the effect of bradykinin or histamine.

DISCUSSION

The results of these studies provide further information about the proteins contained in eosinophils. Because our earlier studies with guinea pig eosinophil granules had identified a basic protein which accounted for better than half of the granule protein (7, 8), we sought evidence for the existence of a similar protein in the human eosinophil. Analysis of lysates of human eosinophil granules by polyacrylamide gel electrophoresis and by gel filtration yielded results virtually identical to those obtained with guinea pig granules and indicated that a MBP is present in the human eosinophil granule. The human MBP has a molecular weight of approximately 9,200, slightly lower than the guinea pig MBP, but like the guinea pig MBP it contains a high content of arginine (12%) and it readily polymerizes to form a series of disulfide-linked polymers. Also, the human MBP is able to cross-react with antiserum to the guinea pig MBP.[2] Finally, neither of the MBP increased vascular

[2] Lewis, D. M., and G. J. Gleich. Unpublished observations.

TABLE II

Amino Acid Composition of Guinea Pig MBP, Human MBP, and CLC

	Guinea pig MBP*	Human MBP		CLC			
		Pt 1	Pt 3	Pt 1	Pt 2	Pt 3	Pt 4
Aspartic	6	7	7	10	9	10	10
Threonine	4	4	4	5	5	5	6
Serine	4	5	4	8	8	9	9
Glutamic	8	7	8	18	18	19	19
Proline	5	2	1	5	5	4	4
Glycine	12	8	8	5	5	5	5
Alanine	8	5	5	4	4	3	4
Valine	9	5	5	13	13	13	13
Methionine	1	0	0	4	4	4	4
Isoleucine	2	4	3	4	4	4	4
Leucine	4	5	6	8	8	9	9
Tyrosine	4	4	4	5	5	5	5
Phenylalanine	5	5	5	7	7	7	7
Histidine	3	2	2	2	2	2	2
Lysine	2	2	3	7	7	8	7
Arginine	13	10	11	6	6	6	6
Tryptophan‡	4	ND§	8	ND	ND	4	3
Cysteine‖	6	ND	6	ND	ND	2	2

* From reference 8.

‡ After hydrolysis in 3 N *p*-toluenesulfonic acid (19).

§ ND, not determined.

‖ As cysteic acid.

permeability in the blued guinea pig, nor did they contract the guinea pig ileum.

Primate eosinophils are remarkable in that they form crystalline substances, the CLC (23). Prior studies of CLC had suggested that they were proteinaceous and of low molecular weight (4–6). CLC were prepared by the method of Archer and Blackwood and crystals with a characteristic dipyramidal structure largely free from contaminating debris formed from the eosinophil homogenates (6). Our studies of the CLC protein indicate that it is composed of a single polypeptide chain with a molecular weight of about 13,000, has a low content of carbohydrate, and has a remarkable tendency to aggregate. The CLC protein contains a high content of glutamic acid and methionine but only six residues of arginine. Solubilized CLC did not increase vascular permeability in guinea pig skin nor contract the guinea pig ileum.

Comparison of the physiochemical properties of the human MBP and the CLC protein indicated that they are distinct substances. This conclusion is based on differences in molecular weight and amino acid composition as well as the observation that two bands were found when mixtures of the purified proteins were analyzed by SDS polyacrylamide gel electrophoresis. Furthermore, both the human and guinea pig MBP readily form disulfide-bonded aggregates while the CLC protein does not. The CLC protein clearly aggregates but evidently on the basis of noncovalent interactions between chains. Finally, the MBP never formed structures resembling CLC.

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