

## Purification of human eosinophil-derived neurotoxin

[Gordon phenomenon/eosinophil granules/major basic protein/Charcot–Leyden crystal protein (lysophospholipase)/Purkinje cells]

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**ABSTRACT** Human and animal eosinophils contain a powerful neurotoxin that causes selective neuronal and axonal damage to white matter of cerebellum and spinal cord of experimental animals when injected intrathecally. This reaction is termed the "Gordon phenomenon." We purified the eosinophil-derived neurotoxin from eosinophil-rich leukocyte suspensions or eosinophil granules from four patients with various hypereosinophilic syndromes. A single protein with an average molecular weight of 18,400 was isolated by sequential chromatography on Sephadex G-50 columns and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis of column fractions. The purified eosinophil-derived neurotoxin from the cells of these patients retained the potent neurotoxic activity of the crude eosinophil or eosinophil granule extracts in experimental animals. These animals developed the syndrome of stiffness and ataxia progressing to severe paralysis characteristic of the Gordon phenomenon. Histologic examination of the brains of animals injected with purified eosinophil-derived neurotoxin confirmed the characteristic widespread loss of Purkinje cells and severe spongiform vacuolation in the white matter of cerebellum, brain stem, and spinal cord. We have established the location of eosinophil-derived neurotoxin in the eosinophil granule and have shown that it is distinct from several other eosinophil proteins, the granule major basic protein, and the Charcot–Leyden crystal protein (lysophospholipase).

Eosinophils contain a powerful neurotoxin that can severely damage myelinated neurons in experimental animals (1–5). M. H. Gordon described this neurotoxic reaction in 1932; it is now known as the "Gordon phenomenon" in his honor. At present, the characteristic and easily recognizable neurologic and histopathologic changes that occur after injection of eosinophil extracts into laboratory animals provide the only available assay for the eosinophil-derived neurotoxin (EDN). The neurologic abnormalities include stiffness and ataxia, progressing to severe paralysis. Histopathologically, there is selective and widespread loss of Purkinje cells (1–7) and severe spongiform degeneration in the white matter of the cerebellum, brain stem, and spinal cord (7).

In a previous study, EDN was partially purified by ultracentrifugation of sonicated human eosinophils followed by fractionation of the supernatant on Sephadex G-50 columns. Fractions with neurotoxic activity eluted at an apparent molecular weight of approximately 15,000. The partially purified material withstood lyophilization and dialysis, but its neurotoxic activity was destroyed by heating at 90°C (7). We now report on the further purification of EDN to homogeneity, establish its molecular weight and origin in eosinophil granules, and distinguish it from other major eosinophil and eosinophil granule components.

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## MATERIALS AND METHODS

**Patients.** Patient 1 had eosinophilic gastroenteritis with peritonitis, and eosinophils were obtained from ascitic fluid as described (7). Patients 2, 3, and 4 were diagnosed as having the hypereosinophilic syndrome. Patient 3 presented with neurologic abnormalities including vertigo, weakness of arms and legs, and disorientation.

**Purification of Eosinophils.** Eosinophils from patient 1 were purified from ascitic fluid that contained a high proportion of eosinophils. Leukocytes from this ascitic fluid were partially purified by centrifugation after layering on Ficoll/Hypaque as described (7, 8). After hypotonic lysis of erythrocytes, followed by three washes in normal saline, the leukocytes were resuspended in isotonic saline at a concentration of  $3.5 \times 10^7$  cells per ml and stored at  $-75^\circ\text{C}$ . This preparation contained 98% eosinophils, 0% neutrophils, and 2% mononuclear cells.

Eosinophils from patients 2, 3, and 4 were purified from eosinophil-rich leukocyte suspensions obtained by cytopheresis of patients on a Haemonetics semi-continuous-flow cell processor (Haemonetics, Natick, MA) (9). The anti-coagulant used was 2% sodium citrate added to 6% hydroxyethyl starch. After sedimentation of erythrocytes, cells in the supernatants were centrifuged at  $200 \times g$  for 5 min and washed once with Dulbecco's phosphate-buffered saline, pH 7.4 ( $\text{P}_i/\text{NaCl}$ ). Residual erythrocytes were lysed by resuspending the pellets in 3 ml of Tris-buffered ammonium chloride, pH 7.2 (10), for every 1 ml of packed cells. After 2 min, the cells were centrifuged at  $200 \times g$ . Cells from patient 2, consisting of 100% eosinophils, were washed twice by centrifugation in  $\text{P}_i/\text{NaCl}$  and used directly to prepare eosinophil granules, major basic protein (MBP), and EDN. Cells from patient 3 were washed twice with  $\text{P}_i/\text{NaCl}$  and used to prepare Charcot–Leyden crystals (CLC) (11) and EDN. A total of  $1.75 \times 10^{11}$  leukocytes was recovered from patient 3, of which 29% or  $5.1 \times 10^{10}$  cells were eosinophils, with 47% neutrophils and 25% mononuclear cells. Eosinophils were not purified further from this sample (see below). Cells from patient 4 were resuspended in 100 ml of RPMI 1640 medium (GIBCO) containing 10% heat-inactivated fetal calf serum (RPMI/fetal calf serum), washed twice with centrifugation, and resuspended in the same medium. A modification of the method of Parillo and Fauci (12) was used to purify eosinophils from this suspension. A total of  $8.6 \times 10^{10}$  cells, containing 47% eosinophils, was applied to 12 nylon wool columns, each containing 6 g of scrubbed nylon wool (Fenwal Laboratories, Deerfield, IL) in 60-ml syringes and equilibrated with RPMI/fetal calf serum at  $37^\circ\text{C}$ . Thirty milliliters of the leukocyte suspension, containing  $2.4 \times 10^8$  cells per ml, was applied to each column, followed

Abbreviations: EDN, eosinophil-derived neurotoxin;  $\text{P}_i/\text{NaCl}$ , phosphate-buffered saline, pH 7.4; MBP, eosinophil granule major basic protein; CLC, Charcot–Leyden crystal.

by incubation at 37°C for 15 min, and cells were eluted with 100 ml of RPMI/fetal calf serum at 37°C. A total of  $5.6 \times 10^{10}$  cells was recovered for an overall eosinophil recovery of 97%. This preparation contained 70% eosinophils, 25% neutrophils, and 5% mononuclear cells.

**Purification of Eosinophil Granules.** Eosinophil granules were prepared from the eosinophil-rich leukocyte suspensions obtained from patients 2 and 4 as described in detail elsewhere (13–15). Briefly, cells were gently washed once with ice-cold 0.25 M sucrose, resuspended in cold 0.25 M sucrose, disrupted by vigorous and repeated pipetting through a narrow-bore 10-ml calibrated pipette, and centrifuged to remove unbroken cells and large cell debris. The pellet was resuspended in 0.25 M sucrose and the extraction procedure was repeated four times. The supernatants from these extractions were centrifuged at  $13,000 \times g$  for 20 min to sediment the granules (Fig. 1). EDN was purified from these eosinophil granules as described below.

**Purification of Eosinophil MBP and CLC Proteins.** The methods for purification of human MBP are described in detail elsewhere (13–15). Briefly, purified eosinophil granules were dissolved in 0.01 M HCl and centrifuged at  $40,000 \times g$  for 5 min, and the supernatant was fractionated on a  $1.2 \times 47$  cm Sephadex G-50 column equilibrated with 0.025 M sodium acetate buffer, pH 4.2/0.15 M NaCl. The fractions from the third protein peak, which contained MBP, were pooled and MBP was stabilized by reduction and alkylation of its two sulfhydryl groups and was dialyzed overnight against 0.15 M NaCl, using 3500  $M_r$  cutoff Spectrapor dialysis casing (Spectrum Medical, Los Angeles). MBP concentrations were determined by absorbance at 277 nm,  $A_{1\text{ cm}}^{1\%} = 26.3$  (15).

The methods for purification of CLC and solubilization of

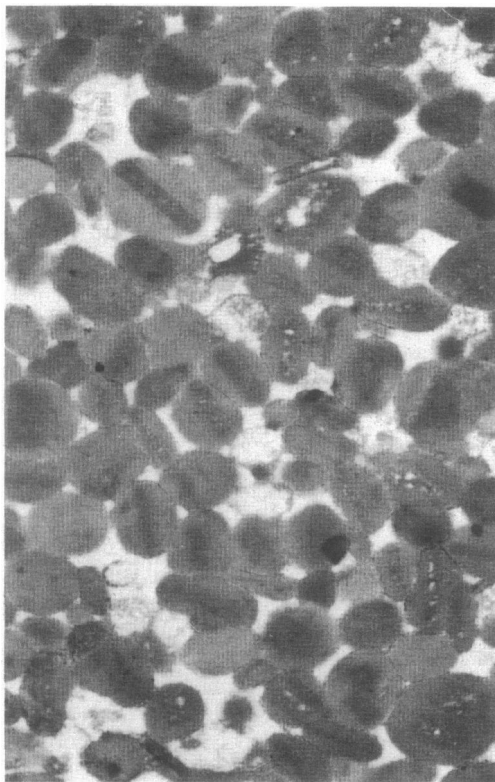


FIG. 1. Transmission electron photomicrograph of purified eosinophil granules from patient 2. ( $\times 19,000$ .) Granules were fixed with 3% phosphate-buffered glutaraldehyde (pH 7.4), postfixated in 1%  $\text{OsO}_4$ , and embedded in Epon, and sections were stained with uranyl acetate and lead citrate. The picture shows the homogeneity of the purified preparation with numerous membrane-bound crystalloid-containing granules with typical electron-dense cores and granular matrix.

CLC protein are described in detail elsewhere (11). Briefly, cells in the eosinophil-rich leukocyte suspension were lysed in hypotonic  $\text{P}_i/\text{NaCl}$  (0.15% NaCl) and repeatedly homogenized in a tissue grinder. The homogenate was centrifuged at  $40,000 \times g$  to remove unbroken cells and insoluble cell debris, and again at  $40,000 \times g$  to yield clear supernatants. Supernatants were frozen slowly at  $-20^\circ\text{C}$  and thawed at  $37^\circ\text{C}$ . The CLC that formed were washed extensively with distilled water until a homogeneous preparation that was free of contaminating debris as assessed by phase-contrast microscopy was obtained. Washed CLC were lyophilized and stored desiccated at  $-20^\circ\text{C}$ . CLC protein for injection was solubilized in  $\text{P}_i/\text{NaCl}$  (11) and protein concentration was determined by absorbance at 277 nm,  $A_{1\text{ cm}}^{1\%} = 12.0$  (15).

**Purification of EDN.** EDN was purified by Sephadex G-50 column chromatography of eosinophil extracts (patients 1 and 3) or eosinophil granule extracts (patients 2 and 4). Eosinophils from patient 1 ( $3.5 \times 10^7$  cells per ml) were thawed from  $-75^\circ\text{C}$  and centrifuged at  $40,000 \times g$  for 30 min to yield a clear eosinophil extract. The supernatant was chromatographed at  $4^\circ\text{C}$  on a  $1.2 \times 47$  cm Sephadex G-50 column equilibrated with 0.025 M acetate buffer, pH 4.2/0.15 M NaCl. Flow rate was 12 ml/hr and 1.0-ml fractions were collected. Fractions containing an 18,000  $M_r$  band by  $\text{NaDodSO}_4$ /polyacrylamide gel electrophoresis were pooled, dialyzed overnight against  $\text{P}_i/\text{NaCl}$ , and concentrated on an Amicon UM-2 membrane. EDN was purified in a similar fashion from the eosinophil extract obtained from patient 3 after the purification of CLC. Eosinophil granule pellets from patient 2 and patient 4 were solubilized in 2 ml of 0.01 M HCl, pH 2.0, and centrifuged at  $40,000 \times g$  for 10 min to yield a clear granule extract, and the  $40,000 \times g$  supernatant was chromatographed at  $4^\circ\text{C}$  on a  $1.2 \times 47$  cm Sephadex G-50 column equilibrated with 0.025 M acetate buffer, pH 4.2/0.15 M NaCl. Flow rate was 12 ml/hr and 1.3-ml fractions were collected. Fractions composing the second protein peak (eluting between 27 and 33 ml in Fig. 2a) were pooled, dialyzed overnight against  $\text{P}_i/\text{NaCl}$ , centrifuged at  $12,000 \times g$  for 10 min, and rechromatographed on a second Sephadex G-50 column equilibrated with  $\text{P}_i/\text{NaCl}$  (Fig. 2b). Fractions eluting between 27 and 33 ml were pooled and concentrated on an Amicon UM-2 membrane.

**$\text{NaDodSO}_4$ /Polyacrylamide Gel Electrophoresis.** Purified eosinophil and eosinophil granule proteins (EDN, MBP, and CLC) were analyzed by electrophoresis in 8% polyacrylamide gels (pH 7.4) containing 1%  $\text{NaDodSO}_4$  by the procedure described by Fairbanks *et al.* (16). Samples were tested with and without reduction by 40 mM final concentration dithiothreitol and gels were stained with Coomassie brilliant blue.

**Bioassay of Eosinophil Proteins for Neurotoxic Activity.** The methods for intrathecal injection of eosinophil extracts and fractions to produce the Gordon phenomenon have been described (7). Briefly, male New Zealand White rabbits weighing 1.5–3.0 kg were sedated with fentanyl and droperidol (Innovar, McNeil Laboratories, Irvine, CA). A 25-gauge needle was passed into the cisterna magna. The correct position was established by withdrawing a small amount of cerebrospinal fluid, and 0.2 or 0.4 ml of test suspension in  $\text{P}_i/\text{NaCl}$  was injected. To eliminate possible bias in recording the results of animal experiments, eosinophil fractions were coded so that the observer did not know which fraction each animal had received. After injection, rabbits were examined daily for the typical signs of the neurotoxic reaction. For histologic study of the brain, rabbits were killed by intravenous injection of pentobarbitone. The brain and spinal cord were immediately removed and fixed in formalin prior to routine embedding, cutting, and staining.

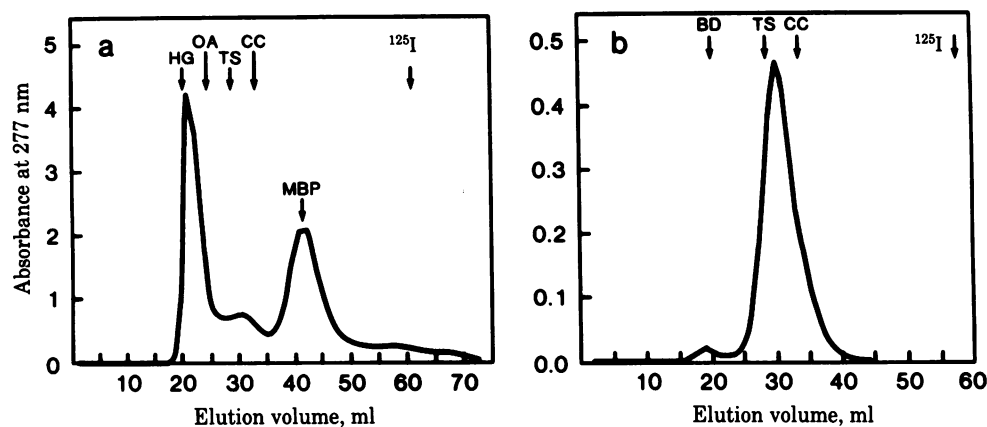


FIG. 2. Sephadex G-50 column chromatography of acid-solubilized eosinophil granules from patient 2. (a) Two milliliters of an acid-solubilized eosinophil granule extract was chromatographed on a Sephadex G-50 column equilibrated with 0.025 M acetate buffer, pH 4.2/0.15 M NaCl. Fractions eluting between 27 and 33 ml (second protein peak) were pooled, dialyzed overnight against  $P_i$ /NaCl, and centrifuged at  $12,000 \times g$  for 10 min. (b) This sample (1.2 ml;  $0.323 A_{277}$  unit/ml) was chromatographed on a second Sephadex G-50 column equilibrated with  $P_i$ /NaCl. Fractions eluting between 27 and 33 ml were pooled and concentrated to 0.6 ml ( $0.129 A_{277}$  unit) on an Amicon UM-2 membrane. Molecular weight markers for the columns included blue dextran (BD;  $>2 \times 10^6 M_r$ ), human gamma globulin (HG;  $150,000 M_r$ ), ovalbumin (OA;  $49,000 M_r$ ), trypsinogen (TS;  $24,000 M_r$ ), cytochrome c (CC;  $12,400 M_r$ ), and  $^{125}I$ .

**RESULTS**

**Assay of Eosinophil and Eosinophil Granule Extracts, MBP, and CLC Protein.** Crude freeze-thaw extracts of eosinophils, eosinophil granules, purified MBP, and CLC protein (lysophospholipase) were injected intrathecally into rabbits to test for neurotoxic activity and the results are shown in Table 1. Typical manifestations of the Gordon phenomenon occurred in two out of three rabbits injected with a crude freeze-thaw extract of purified eosinophils from patient 1 in 7-8 days. A freeze-thaw extract of purified eosinophil granules (Fig. 1) from patient 2 produced the Gordon phenomenon in two rabbits in 3-6 days. In contrast, injection of highly purified MBP from two different patient sources, CLC protein from patient 3, eosinophil granule enzymes (void volume peak eluting between 20 and 25 ml from the Sephadex G-50 column shown in Fig. 2a) and human serum albumin as controls failed to induce neurotoxic reactions.

**Purification of EDN.** The above results indicated that EDN activity was not associated with CLC, eosinophil granule enzymes, and MBP. Because the latter substances constitute the major protein peaks found by gel filtration of eosinophil granule extracts on Sephadex G-50 (peaks 1 and 3 in Fig. 2a), we suspected that EDN activity resided in the second protein peak (eluting between 27 and 33 ml in Fig. 2a). Therefore, these fractions were concentrated and rechromatographed on a second Sephadex G-50 column at pH 7.4 as shown in Fig. 2b. Analysis of concentrated fractions from the major peak by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis showed a band (Fig. 3b) with a molecular weight of approximately 18,000. Injection of this purified material produced a neurotoxic reaction in rabbits (Table 1, EDN from patient 2).

We subsequently purified EDN from freeze-thaw extracts of human eosinophils (patients 1 and 3) and from acid extracts of purified eosinophil granules (patient 4) as described in *Materials and Methods*. These preparations retained the potent neurotoxic activity of the crude eosinophil and eosinophil granule extracts (Table 1). NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis analysis of purified EDN from patient 1 (Fig. 3a) and patient 4 (results not shown) revealed a single protein band with a molecular weight of approximately 18,000. The partially purified EDN from the eosinophil extract of patient 3 was heterogeneous and contained three protein bands (results not shown), one of which had a molecular weight of approximately 18,000. The mean molecular weight ( $\pm$  SEM) for purified EDN was  $18,400 \pm 300$  (Fig. 4).

**Neurologic Manifestations.** The neurologic abnormalities observed in rabbits injected with purified EDN or crude eosinophil or eosinophil granule extracts developed along a predictable course (1-7). The initial signs included stiffness, most pronounced in the forelimbs, followed by incoordination and ataxia that progressed until the animals could not remain upright. The final phase of the Gordon phenomenon was characterized by

Table 1. Incidence of neurotoxic reaction (Gordon phenomenon) in rabbits injected intrathecally with various crude extracts and purified proteins of human eosinophils and eosinophil granules

Material injected*	Source of eosinophils	Average onset of symptoms, days	No. rabbits with Gordon phenomenon/total
Crude eosinophil extract (freeze-thaw) <sup>†</sup>	Patient 1	7.5	2/3
Crude eosinophil granule extract (freeze-thaw) <sup>‡</sup>	Patient 2	4.5	2/2
Eosinophil granule enzymes (Sephadex G-50 peak I; Fig. 2a)	Patient 2	—	0/2
MBP	Patient 2 <sup>§</sup>	—	0/6
MBP	Patient 2	—	0/2
MBP	Patient 4	—	0/4
CLC protein	Patient 3	—	0/6
Purified EDN <sup>¶</sup>	Patient 1	8.0	2/2
Purified EDN	Patient 2	4.5	2/2
Purified EDN	Patient 4	3.0	3/3
Partially purified EDN	Patient 3 <sup>¶</sup>	8.3	3/3
Human serum albumin	—	—	0/4

\* Animals were injected with 50  $\mu$ g of test material in 0.4 ml of  $P_i$ /NaCl.

<sup>†</sup> This sample produced the Gordon phenomenon in 4/4 (positive/total) rabbits when injected intracerebrally and 12/12 when injected intrathecally as previously reported (7).

<sup>‡</sup> Protein concentration determined by the biuret reaction.

<sup>§</sup> Test material consisted of 0.2 ml containing 10  $\mu$ g of MBP.

<sup>¶</sup> Protein concentration estimated by absorbance at 277 nm, assuming  $A_{1\text{cm}}^{1\%} = 10.0$

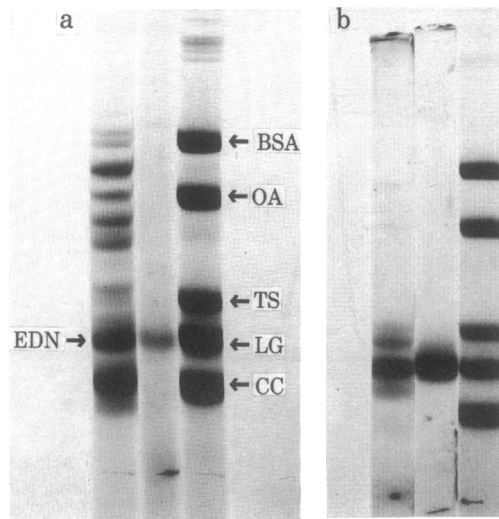


FIG. 3. NaDodSO<sub>4</sub>/8% polyacrylamide gel electrophoresis analysis of reduced samples of purified EDN (center gels) from patient 1 (a) and patient 2 (b). Gels on the left show either the crude eosinophil extract (a) or partially purified EDN prior to final Sephadex G-50 column fractionation (b). Gels on the right show the molecular weight protein standards, which included certain of those used in Fig. 2 plus bovine serum albumin (BSA; 69,000  $M_r$ ), and  $\beta$ -lactoglobulin (LG; 18,000  $M_r$ ). The stab mark in the bottom of the gel indicates the distance traveled by the pyronin-Y marker.

severe weakness and muscle wasting. Some animals developed nystagmus and jerky, repetitive head movements. No evidence of neurologic abnormalities of higher-level functions were observed; even severely affected animals remained alert and

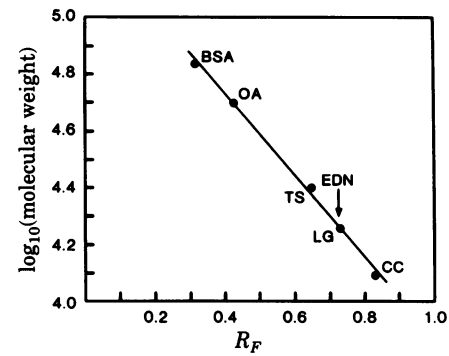


FIG. 4. Molecular weight determination for EDN by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis. EDN from patient 1 and molecular weight markers (see captions to Figs. 2 and 3) were reduced with dithiothreitol and electrophoresed in 8% polyacrylamide gels. Relative migration values ( $R_F$ ) are presented as a ratio of the distance migrated for each protein relative to the distance migrated by the pyronin-Y tracking dye. The molecular weight of EDN was calculated from the equation for the least squares regression line ( $r^2 = 0.999$ ). The mean molecular weight ( $\pm$ SEM) of EDN from the four patients was 18,400  $\pm$  300 ( $n = 7$ ).

would eat and drink in a normal fashion if food and water were within reach. The latent period between injection of purified EDN and the onset of neurologic manifestations ranged between 3 and 11 days (Table 1).

**Histopathologic Changes.** On light microscopy, the histologic abnormalities found in rabbits injected with purified EDN were concentrated in cerebellum, pons, and spinal cord. The disappearance of Purkinje cells from the cerebellum that is a hallmark of the Gordon phenomenon was a constant pathologic

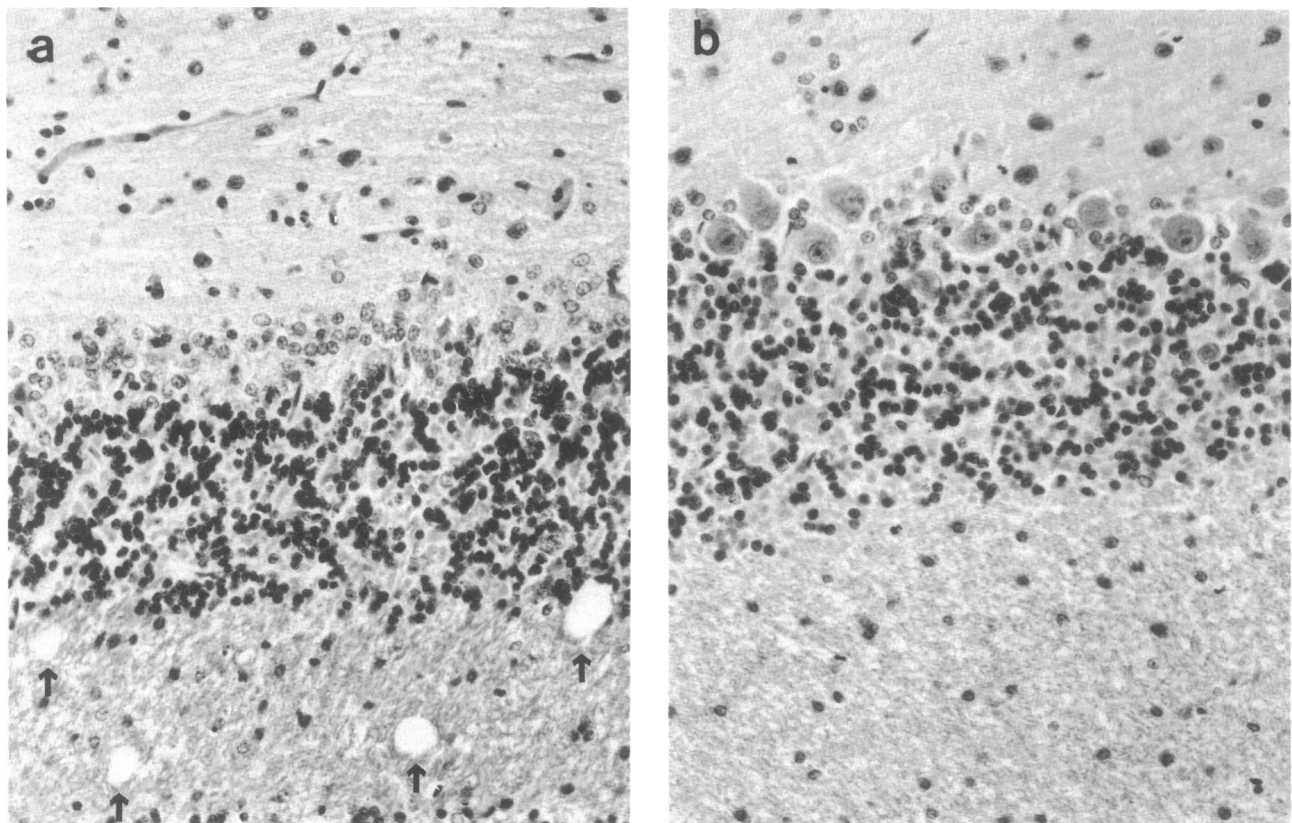


FIG. 5. Histopathologic abnormalities. (a) Section of cerebellum from a rabbit injected with 50  $\mu$ g of EDN purified from the eosinophil granules of patient 2, showing characteristic loss of Purkinje cells and spongiform vacuolation of the white matter (arrows). (Hematoxylin and eosin,  $\times 250$ .) (b) Section of cerebellum from a rabbit injected with 50  $\mu$ g of CLC protein purified from the eosinophils of patient 3. In this case, as well as after injection of 50  $\mu$ g of MBP, the cerebellum appeared normal. Note the large Purkinje cells. (Hematoxylin and eosin,  $\times 250$ .)

observation in affected rabbits (Fig. 5a). The white matter of cerebellum, pons, and spinal cord showed gross spongiform changes (7). There was no inflammatory reaction apart from the presence of macrophages and the grey matter was normal. In contrast, these tissues appeared normal in rabbits injected with MBP or CLC protein (Fig. 5b).

### DISCUSSION

Human and animal eosinophils contain a powerful neurotoxin that causes neuronal and axonal damage in the form of a loss of Purkinje cells (1-7) and a severe spongy vacuolation of the white matter of cerebellum, brain stem, and spinal cord (7). Durack *et al.* (7) showed that neurotoxic activity eluted from Sephadex G-50 with an apparent molecular weight of approximately 15,000. We have confirmed and extended these observations by purifying EDN to homogeneity from eosinophils and purified eosinophil granules from three patients with various hypereosinophilic syndromes. We have established the molecular weight of EDN and distinguished it from several other major eosinophil components, including MBP and CLC protein (lysophospholipase), and eosinophil enzymes such as peroxidase.

These studies demonstrate that EDN is located in the eosinophil granule. Previously, Seiler *et al.* (6) found histopathologic changes characteristic of the Gordon phenomenon in guinea pigs injected with a mixture of mitochondria, eosinophil granules, and mast cell granules prepared from rat peritoneal exudate cells. On this evidence, they suggested that the neurotoxin was located in eosinophil granules. In the present study, both a freeze-thaw extract of highly purified eosinophil granules obtained from human peripheral blood eosinophils and purified EDN from these same granules retained the potent neurotoxic activity of a crude eosinophil extract.

The demonstration that EDN is located in eosinophil granules invites comparison of its properties with those of other granule-derived proteins. MBP damages cells (17-19) and parasites (19-21). Therefore, MBP was a likely candidate for the Gordon phenomenon. Our demonstration that human MBP has a molecular weight (9300) nearly half that of EDN (18,400), that it can be separated from EDN by gel chromatography, and that it does not produce the Gordon phenomenon when injected intrathecally into rabbits establishes that MBP is not the agent responsible for the Gordon phenomenon. The finding that the eosinophil granule enzymes were inactive excludes peroxidase as the cause of the Gordon phenomenon. Also, EDN is distinct from CLC protein on the basis of molecular weight and the failure of purified CLC protein to induce a neurotoxic reaction in animal experiments. Weller *et al.* (22) have recently identified the CLC protein as lysophospholipase (lysolecithin acylhydrolase, EC 3.1.1.5), and results of the present study indicate that intrathecal injection of the purified enzyme does not induce the Gordon phenomenon or any other neurotoxic pathology. Finally, EDN is not likely to be the eosinophil cationic protein (23) because they differ in molecular weight (18,400 versus 21,000, respectively) and because purified EDN contained less than 0.1% eosinophil cationic protein by radioimmunoassay (unpublished observations).<sup>‡</sup>

The biological and clinical significance of the Gordon phenomenon remains enigmatic. Certainly, eosinophil granules contain a powerful neurotoxin that is capable of damaging and ultimately destroying myelinated axons and neurons. The dam-

age is highly selective in the experimental animal, affecting chiefly white matter of cerebellum and spinal cord. Lesions may occur elsewhere in the brain and peripheral nerves, but these are minor compared with the destruction of white matter in cerebellum and spinal cord. The neurotoxic effect is dose dependent. If a high dose is given, severe destructive changes develop within 2 or 3 days. Lower doses cause less severe damage, with a longer latent period until onset of symptoms. In man, varied neurologic abnormalities, including weakness, paralysis, incoordination, and cranial nerve lesions, have been reported as common findings in patients with idiopathic hypereosinophilic syndrome (24), cerebrospinal fluid eosinophilia (25), and eosinophilic meningoencephalitis associated with helminthic parasites (26, 27). However, no stereotyped syndrome like the Gordon phenomenon has been recognized in man. Indeed, patient 3 in the present study had neurologic abnormalities in association with the hypereosinophilic syndrome. The Gordon phenomenon in experimental animals may, therefore, have its counterpart in human diseases characterized by tissue or peripheral blood eosinophilia and neurologic involvement.

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- Gordon, M. H. (1933) *Br. Med. J.* **1**, 641-647.
- King, L. S. (1939) *J. Exp. Med.* **70**, 303-304.
- McNaught, J. B. (1938) *J. Am. Med. Assoc.* **111**, 1280-1284.
- Turner, J. C., Jackson, H., Jr. & Parker, F., Jr. (1938) *Am. J. Med. Sci.* **195**, 27-33.
- Schwartz, A. M., Lapham, L. W. & Van den Noort, S. (1966) *Neurology* **16**, 1118-1126.
- Seiler, G., Westerman, R. A. & Wilson, J. A. (1969) *Neurology* **19**, 478-488.
- Durack, D. T., Sumi, S. M. & Klebanoff, S. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1443-1447.
- Boyum, A. (1968) *Scand. J. Clin. Lab. Invest. Suppl.* **97**, 1-109.
- Pineda, A. A. S., Brzica, M., Jr. & Taswell, H. F. (1977) *Transfusion* **17**, 407-416.
- Boyle, W. (1968) *Transplantation* **6**, 761-764.
- Ackerman, S. J., Loegering, D. A. & Gleich, G. J. (1980) *J. Immunol.* **125**, 2118-2126.
- Parillo, J. E. & Fauci, A. S. (1978) *Blood* **51**, 457-473.
- Gleich, G. J., Loegering, D. A. & Maldonado, J. E. (1973) *J. Exp. Med.* **137**, 1459-1471.
- Gleich, G. J., Loegering, D. A., Kueppers, F., Bajaj, S. P. & Mann, K. G. (1974) *J. Exp. Med.* **140**, 313-332.
- Gleich, G. J., Loegering, D. A., Mann, K. G. & Maldonado, J. E. (1976) *J. Clin. Invest.* **57**, 633-640.
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606-2617.
- Gleich, G. J., Frigas, E., Loegering, D. A., Wassom, D. L. & Steinmuller, D. (1979) *J. Immunol.* **123**, 2925-2927.
- Frigas, E., Loegering, D. A. & Gleich, G. J. (1980) *Lab. Invest.* **42**, 35-43.
- Butterworth, A. E., Wassom, D. L., Gleich, G. J., Loegering, D. A. & David, J. R. (1979) *J. Immunol.* **122**, 221-229.
- Wassom, D. L. & Gleich, G. J. (1979) *Am. J. Trop. Med. Hyg.* **28**, 860-863.
- Kierszenbaum, F., Ackerman, S. J. & Gleich, G. J. (1981) *Am. J. Trop. Med. Hyg.*, in press.
- Weller, P. F., Goetzl, E. J. & Austen, K. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7440-7443.
- Olsson, I., Venge, P., Spitznagel, J. K. & Lehrer, R. I. (1977) *Lab. Invest.* **36**, 493-500.
- Chusid, M. J., Dale, D. D., West, B. C. & Wolff, S. M. (1975) *Medicine* **54**, 1-27.
- Snead, O. C. & Kalavsky, S. M. (1976) *J. Pediatr.* **89**, 83-84.
- Terplan, K., Kraus, R. & Barnes, S. (1957) *J. Mt. Sinai Hosp.* **24**, 1293-1309.
- Yii, C.-Y. (1976) *Am. J. Trop. Med. Hyg.* **25**, 233-249.

<sup>‡</sup> Preliminary studies conducted in collaboration with P. Venge and I. Olsson indicate that MBP and the eosinophil cationic protein are distinct entities.