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Hypodense eosinophils and interleukin 5 activity in the blood of patients with the eosinophilia–myalgia syndrome

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ABSTRACT **The recent recognition of the eosinophilia–myalgia syndrome (EMS) associated with the ingestion of L-tryptophan prompted an analysis of the peripheral blood eosinophil phenotypes and of the serum eosinophil hematopoietins in this disorder. Five patients with an illness characterized by the abrupt onset of aching skeletal muscles, edema, thickening and induration of the skin, and marked blood eosinophilia associated with L-tryptophan ingestion provided eosinophils, serum, or both, for evaluation. Gradient sedimentation density analysis of the peripheral blood eosinophils from four of these patients revealed that 43 ± 13% (mean ± SEM) of the cells had converted to the abnormal (hypodense) sedimenting phenotype. When normodense eosinophils from the reference donors were cultured for 3 days in medium supplemented with increasing concentrations of serum from the patients with EMS, their viability increased in a dose-dependent manner to 45%, which was significantly augmented over the effect of normal serum. This eosinophil viability-sustaining activity was inhibited by 76 ± 7% (mean ± SEM; $n = 3$) by the addition of anti-interleukin 5 (IL-5) but not by neutralizing antibodies monospecific for either granulocyte/macrophage colony-stimulating factor (GM-CSF) or IL-3. IL-5, an eosinophilopoietic factor, converts normodense peripheral blood eosinophils *in vitro* to a hypodense sedimenting form with extended viability and augmented biologic responses to activating stimuli. Thus, the presence of IL-5 in the sera of patients with EMS may contribute to the development and maintenance of the eosinophilia and may regulate the conversion of the peripheral blood eosinophils to the hypodense phenotype with augmented pathobiologic potential.**

The eosinophilia–myalgia syndrome (EMS), associated with the ingestion of L-tryptophan, is characterized by peripheral blood eosinophilia, myalgia, thickened and indurated skin, and peripheral edema (1–7). The histologic changes include edema, cellular infiltration, and fibrosis of the dermis, fascia, and muscle. The perivascular infiltration by lymphocytes, plasma cells, and eosinophils is accompanied by the deposition of eosinophil granule proteins (6). Despite the prominence of the peripheral blood eosinophilia in EMS, the phenotype of the eosinophils and the putative cytokine(s) responsible for the development of the eosinophilia have not been determined.

Eosinophils isolated from the peripheral blood of patients with the idiopathic hypereosinophilic syndrome (IHES), asthma, and helminthic infections have a lower mean gradient sedimentation density (hypodense) than that of eosinophils purified from healthy individuals (normodense) (8–12). In comparison with normodense eosinophils, hypodense eosinophils generate increased amounts of leukotriene C_4 (LTC_4) in response to stimulation with calcium ionophore A23187 and have an augmented capacity to mediate antibody-dependent cytotoxicity (10, 11). Normodense eosinophils cultured *in vitro* with picomolar concentrations of granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL-3), or interleukin 5 (IL-5) maintain their viability for at least 14 days and are converted to a hypodense phenotype that is primed for enhanced calcium ionophore-initiated LTC_4 generation and antibody-mediated cytotoxicity (13–15). The presence of IL-5 in the serum of patients with IHES suggests that this hematopoietin plays a regulatory role when some of the peripheral blood eosinophils express the hypodense phenotype with augmented viability and function (16). We studied the eosinophil phenotypes and associated cytokine activities in the blood of patients with EMS.

MATERIALS AND METHODS

Patients. Five Caucasian females with a mean ± SEM age of 55 ± 7 years who ingested L-tryptophan for a mean ± SEM period of 19 ± 7 months in usual doses ranging from 500 to 2000 mg/day (mean ± SEM, 1280 ± 252) were identified with peripheral blood eosinophilia, a clinical course consistent with the recently described EMS, and a history of L-tryptophan ingestion (Table 1). Their charts were reviewed retrospectively, and the patients were followed as clinically indicated. All patients were examined by at least one of the authors (R.J.A.).

Myalgia, which was usually abrupt in onset and severe, caused subjective weakness. Arthralgia, without joint warmth or swelling, occurred in the shoulders, wrists, knees, and ankles. Paresthesias involved the forearms and legs in two patients, and the hands and feet in one. Pruritus was generalized. Other concomitant symptoms or signs among the individual patients included alopecia, dry cough, fatigue, lightheadedness, morning stiffness, low grade fever, discomfort at a site of prior incision and radiation therapy, shortness of breath, dry mouth, dysphagia, and an influenza-like prodrome.

On physical examination, skin thickening and induration involved the calves and the medial aspect of the upper inner arms. The chest, abdomen, and thighs were also involved in two patients, and the hands in only one. When present, macular erythema overlay the regions of skin thickening. Shoulder and/or hip range of motion was mildly limited in all patients except one.

All patients had peripheral blood eosinophilia (Table 2). Creatine phosphokinase values were below normal in all patients but patient 4, whose value was normal. Lactate dehydrogenase was six times the upper limit of normal in

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Abbreviations: EMS, eosinophilia–myalgia syndrome; IL-3 and IL-5, interleukins 3 and 5; GM-CSF, granulocyte/macrophage colony-stimulating factor; IHES, idiopathic hypereosinophilic syndrome.

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Table 1. Characteristics of patients with EMS

Patient				L-Tryptophan			
				Dose, mg/day		Duration,	
No.	Age, yr	Sex	Medical history	Usual	Peak	mo	Medications
1	82	F	Hypertension Coronary artery disease Hypercholesterolemia Herpes zoster	500	500	36	Diltiazem Terfenadine Propranolol Isosorbide dinitrate
2	46	F	Asthma Anxiety/depression Rhinitis	1000	1000	38	Prednisone Theophylline Albuterol inhaler Diphenhydramine
3	44	F	Hypertension Erythema chronica migrans	2000	2500	7	None
4	55	F	Atrial septal defect Mitral valve prolapse Depression	1500	5000	12	Clonazepam
5	48	F	Breast carcinoma, stage I Lumpectomy/radiation Chemotherapy Hypothyroidism Allergic rhinoconjunctivitis	1400		3	Levothyroxine

patient 5, but was normal or minimally elevated in the others. The serum aldolase level was within the normal range for all patients. The platelet count was normal in all patients except patient 2, in whom it was 577,000/μl. A sample of bone marrow obtained from patient 1 revealed an increase in eosinophil precursors, but no other abnormalities. Echocardiography in this patient, an 82-year-old woman with congestive heart failure coincident with the onset of EMS, showed global left ventricular hypokinesis with severe mitral regurgitation, left atrial enlargement, fibrosis of the mitral annulus, and thickening of the aortic leaflets; there was no specific evidence for endomyocardial involvement. The congestive heart failure cleared with resolution of her eosinophilia.

Biopsies were obtained of skin and subcutaneous tissue in all patients, of the deep (muscle) fascia in four (patients 2–5), and of skeletal muscle in three (patients 2–4). The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin; selected blocks were also stained with Masson trichrome and/or Giemsa. Eosinophil, lymphohistiocyte, and plasma cell infiltrates were evaluated according to the site (dermis, subcutis, deep fascia, skeletal muscle), and particular attention was paid to the presence of perineurial, endoneurial, perimysial, and endomysial infiltrates and to the extent of myofiber atrophy. A few plasma cells were seen in three patients at one or more tissue sites. Perineurial cellular infiltration was present in all patients at one or more sites, and endoneurial cellular infiltration was present in one (patient 5). Muscle biopsy revealed endomysial and perimysial cellular infiltration without necrosis or regeneration. Eosinophils were present on biopsy in four patients and were located in one or more tissue sites. In three patients, the eosinophilic infiltrate was modest, and it was moderate in one patient. Endothelial cell hypertrophy and lymphohistiocytic and plasma cell infiltration of the venule wall without necrosis or thrombosis was seen in two patients (4 and 5). Early or mild fibrosis was identified by fibroblastic proliferation with edema and minimal collagen deposition, intermediate fibrosis by the presence of well-formed collagen fibers, and advanced (late) fibrosis by dense, paucicellular collagen deposition. Fibrosis was early in three, intermediate in one, and late in one; it occurred in areas of inflammatory cell infiltration and edema.

Eosinophil Isolation and Culture. Eosinophils were isolated from the peripheral blood of seven reference donors who were healthy or were diagnosed with allergic rhinitis and/or asthma and from four of the patients with EMS by the centrifugation of individual dextran (BDH)-sedimented erythrocyte/leukocyte preparations on discontinuous gradients of metrizamide (Nygaard, Oslo) of 18–24% (wt/vol) (13). Eosinophils recovered from the 22/23% and 23/24% metrizamide interfaces and the cell pellet were designated normodense. Eosinophils from all other metrizamide interfaces were designated hypodense. Initial viability was >98% as assessed by the exclusion of trypan blue (GIBCO).

Freshly isolated eosinophils (10^5 cells) were suspended in 200 μl of enriched medium [RPMI 1640 medium supplemented with 100 units of penicillin, 100 μg of streptomycin, and 10 μg of gentamicin per ml, 2 mM L-glutamine, 0.1 mM nonessential amino acids, and 10% (vol/vol) fetal calf serum] alone or in medium supplemented with 10 pM GM-CSF, 10 pM IL-3, 1 pM IL-5 (provided by Genetics Institute, Cambridge, MA), or defined concentrations of human serum (16). For some experiments, various dilutions of a rabbit neutralizing polyclonal antibody against human GM-CSF (Genzyme) or against human IL-3 (Genzyme), a rat monoclonal antibody against mouse IL-5 (purified from clone TRFK-5

Table 2. Clinical findings in patients with EMS

Pt.	Myalgia/ arthralgia	Rash/ pruritus	Paresthesia/ hyperesthesia	Skin thickening	Macular erythema	Edema	Peak eosinophil count, cells/μl
1	+/−	+/+	+/+	+	−	+	5,215
2	+/+	+/−	+/−	+	+	−	4,192
3	+/+	+/−	+/−	+	+	−	3,200
4	+/−	−/−	−/+	−	−	−	3,872
5	−/+	+/+	−/−	+	−	+	12,168

Pt., patient; +, present; −, absent.

generously provided by Robert Coffman of DNAX) conjugated to cyanogen bromide-activated Sepharose CL-4B beads (Sigma), or bovine serum albumin conjugated to Sepharose beads were preincubated for 120 min at 37°C with enriched medium with or without added cytokine or human serum. In experiments with anti-IL-5, the beads were sedimented by centrifugation of the incubation mixture at 250 × *g* for 5 min at 4°C, and the supernatant was used for subsequent experiments. Cell viability was determined by trypan blue exclusion after 72 hr of culture in 96-well, flat-bottom microtiter plates at 37°C in a 5% CO_2/95% air atmosphere.

The statistical significance for the difference in the viability of eosinophils cultured with serum from individual patients with EMS and with serum from individual reference donors was determined by the two-tailed Student *t* test.

RESULTS

Sedimentation Gradient Profile of Eosinophils. Eosinophils isolated from the reference donors were predominantly normodense; only 9 ± 4% were hypodense (mean ± SEM; n = 7) (Fig. 1*A*). The normodense eosinophil gradient fractions from the reference donors contained 82 ± 15% eosinophils and 18 ± 12% neutrophils. Of the eosinophils isolated from patients 1–4 with EMS, 43 ± 13% (mean ± SEM; $P < 0.05$) were hypodense (Fig. 1*B*); these fractions contained 20 ± 8% eosinophils, 55 ± 12% neutrophils, and 25 ± 15% mononuclear cells. The normodense eosinophil gradient fractions from these same four patients with EMS contained 89 ± 9% eosinophils and 11 ± 5% neutrophils.

Viability-Sustaining Activity in Serum from Patients with EMS and Its Neutralization with Antibody. Freshly isolated normodense eosinophils from reference donors were cultured in enriched medium supplemented with increasing concentrations of serum from patients with EMS or from normal reference donors. Supplementation of the enriched medium with serum from individual patients with EMS (patients 1–3 and 5) caused a significant dose-dependent enhancement of eosinophil viability after 72 hr of culture (Fig. 2) as compared with the negligible action of serum from the normal reference donors. Supplementation of the enriched medium with serum from a patient with steroid-unresponsive IHES increased eosinophil viability to 75% as compared with a mean viability of 45% when the medium was supplemented with the same dilution of serum from patients with EMS.

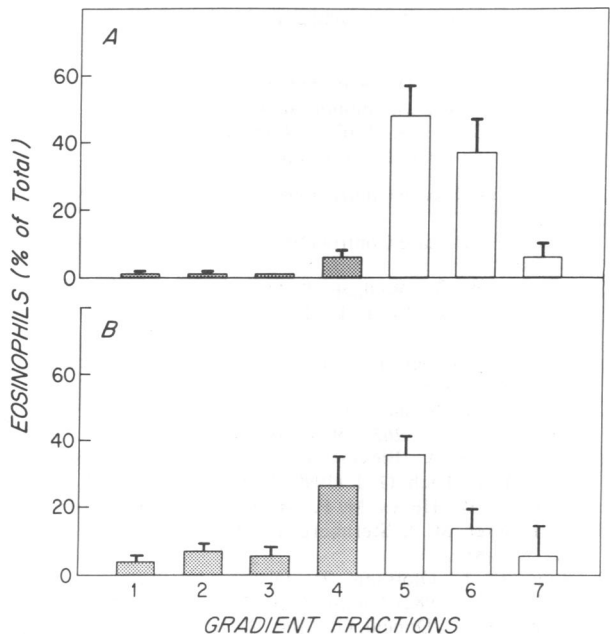

FIG. 1. Density gradient distribution of freshly isolated eosinophils from seven reference donors (*A*) and from four patients with EMS (*B*). Discontinuous metrizamide gradient fractions 1–6 refer to the eosinophils recovered at the 0/18%, 18/20%, 20/21%, 21/22%, 22/23%, and 23/24% metrizamide interfaces, respectively; fraction 7 refers to the eosinophils in the cell pellet and 24% metrizamide. The results are expressed as mean ± SEM. Eosinophils sedimenting in fractions 5–7 are normodense, and those sedimenting in fractions 1–4 are hypodense.

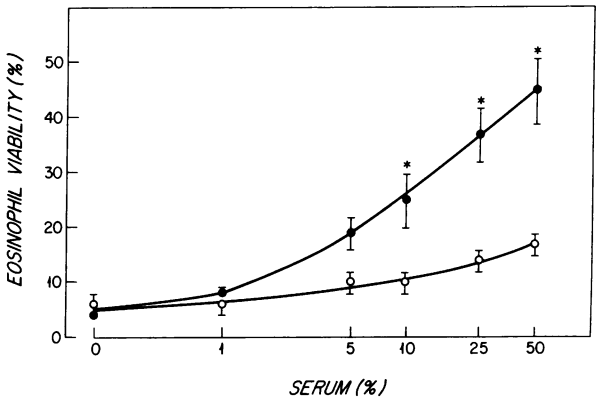

FIG. 2. Effect of serum from patients with EMS on eosinophil viability *ex vivo*. Normodense eosinophils from the reference donors were cultured for 72 hr in enriched medium supplemented with increasing concentrations of serum from four individual patients with EMS (●) or from four individual reference donors (○). Data are expressed as the mean ± SEM. The viability of eosinophils maintained in enriched medium supplemented with sera from patients with EMS was compared statistically with that of replicate eosinophils maintained in medium supplemented with the same concentrations of sera from the reference donors. *, $P < 0.05$.

Anti-IL-3 and anti-GM-CSF completely neutralized the viability-sustaining activity of 10 pM IL-3 and 10 pM GM-CSF at a dilution of ≤1:5000 and at a concentration of ≥5 μg/ml, respectively. At a standard dilution of 1:250 for anti-IL-3 and at concentrations of 100 μg/ml for anti-GM-CSF and 20 μg/ml for anti-IL-5, no cross-neutralizing activity was noted, as defined by inhibition of the viability-sustaining activity for eosinophils maintained with the appropriate cytokine. Anti-IL-5 neutralized the viability-sustaining activity of 1 pM human IL-5 in a dose-dependent manner, and complete neutralization occurred at concentrations of ≥1.0 μg/ml. Bovine serum albumin-Sepharose at concentrations as great as 100 μg/ml had no effect. Preabsorption of 50% serum from patients 1, 2, and 5 with 20 μg of anti-IL-5 diminished mean eosinophil viability after 72 hr of culture from 51 ± 4% to 18 ± 4% ($P < 0.05$) (Fig. 3), a net inhibition of 75%. Neither anti-GM-CSF nor anti-IL-3 reduced the viability of normodense eosinophils maintained for 72 hr in enriched medium supplemented with 50% serum from the patients with EMS. The viability of replicate normodense eosinophils maintained in enriched medium alone was 7 ± 1% (mean ± SEM; n = 3).

DISCUSSION

Five patients with an illness characterized by the abrupt onset of myalgia associated with edema, thickening and induration of the skin, and peripheral blood eosinophilia associated with the ingestion of L-tryptophan were considered to have the recently described EMS (1–7). Macular erythema, when present in these patients, was superimposed on the thickening and induration of the affected skin. Paresthesias and hyperesthesia were common, but axonal neur-

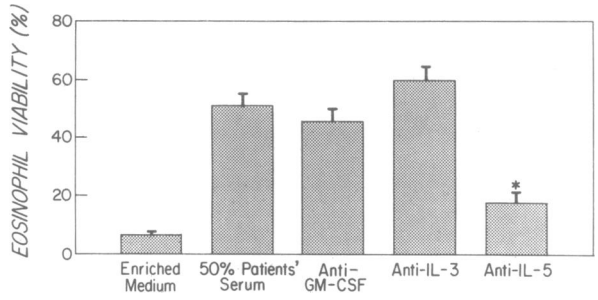

FIG. 3. Effect of antibodies against GM-CSF, IL-3, and IL-5 on the eosinophil viability-sustaining activity of serum from patients with EMS. Normodense eosinophils from reference donors were cultured for 72 hr in enriched medium alone or in medium supplemented with 50% serum from three individual patients with EMS. Replicate serum samples were preincubated with antibodies against GM-CSF, IL-3, and IL-5 as described in the text. Data are expressed as the mean ± SEM (n = 3). *, P <0.05 for the difference in the viability of eosinophils cultured with 50% patients' serum and with 50% patients' serum pretreated with anti-IL-5.

opathy with motor weakness was not seen. In addition to the marked eosinophilia, the patients exhibited depressed creatine phosphokinase activity as noted in earlier reports (4) but no concurrent elevation of serum aldolase activity. There appeared to be no internal organ involvement, with the possible exception of reversible heart failure in patient 1. On biopsy, the dermis, subcutaneous tissue, fascia, and even muscle showed infiltration with mononuclear cells and some eosinophils in varying proportions with mild to moderate fibrosis, and perineurial, endoneurial, endomysial, perimysial, and endovascular infiltrations.

The sedimentation density gradient profile of the peripheral blood eosinophils from the patients with EMS revealed a shift to the hypodense phenotype as compared with eosinophils from reference donors (Fig. 1). The transition fraction of 21/22% was included in defining the hypodense population because the distribution profile was different for the two groups. Because this phenotypic change occurs to a greater degree in the peripheral blood eosinophils from patients with IHES (16), or *ex vivo* in normodense eosinophils from normal donors exposed for 7 days to picomolar concentrations of GM-CSF, IL-3, or IL-5 (13–15), the eosinophils in this fraction were not used previously by our group in defining hypodensity. The serum of patients with EMS elicited a significant dose-dependent increase in the *ex vivo* viability of eosinophils of normal donors as compared with the negligible effect of other normal human serum (Fig. 2). A neutralizing antibody monospecific for IL-5 markedly attenuated the eosinophil viability-sustaining activity in the serum from the three patients with EMS studied (Fig. 3), as observed also for three patients with steroid-unresponsive IHES (16). These data suggest that IL-5, the major eosinophil hematopoietin in the serum of patients with EMS, elicits the eosinophilia and mediates the phenotypic conversion to the hypodense sedimenting state.

The pathogenesis of EMS remains unclear. A toxic contaminant has been suspected (17) but would provide only a partial explanation because the preparation of L-tryptophan consumed by three of our patients was ingested at comparable doses by other individuals who remained asymptomatic. These findings, like those obtained by others (7), suggest that adverse exposure must occur in susceptible individuals. The ingestion of 1 g of L-tryptophan by patients with active EMS, as compared with patients with EMS in whom the eosinophilia has resolved or with normal individuals, results in a marked rise in plasma levels of L-kynurenine and quinolinic acid (7), metabolites of L-tryptophan that are neurotoxic substances (18–25). These findings suggest an increase in activity of indoleamine 2,3-dioxygenase in the presence of increased substrate load. This mechanism has been proposed in a report of a scleroderma-like illness developing in an individual ingesting 5-hydroxytryptophan and carbidopa in whom the peripheral eosinophil count was 1885 cells per mm^3, and plasma levels of serotonin and L-kynurenine were also elevated (26).

The elevated serum and urine levels of eosinophil major basic protein and eosinophil-derived neurotoxin in patients with EMS suggest ongoing eosinophil degranulation (6) as an alternative or concurrent pathobiologic process. Although eosinophils are not prominent histologically in affected organs, eosinophil-granule major basic protein has been found in several organs of a patient with EMS (6). Eosinophil granule proteins are known to be cytotoxic to a variety of mammalian tissues (27), including peripheral nerve cells (28). The ability of IL-5 alone to induce eosinophil degranulation in the absence of a ligand and to greatly enhance ligand-stimulated eosinophil degranulation (29) suggests that this cytokine may contribute to the occurrence of tissue degranulation in EMS. The combined effects of tissue ischemia secondary to micropathic angiopathy and of eosinophil-derived neurotoxins have been considered to be of pathobiologic significance (30).

In summary, IL-5, the predominant eosinophilopoietic factor in the serum of patients with EMS, may contribute to the tissue injury by expanding the peripheral blood population of hypodense eosinophils that are primed for augmented biologic responses to activating stimuli. The finding that IHES and EMS both are characterized by greatly increased serum levels of IL-5 and phenotypically altered peripheral blood eosinophils but differ by the relatively diminished involvement of visceral organs in EMS suggests that local factors determine the tissue distribution of the eosinophils. The distribution of these primed, phenotypically altered eosinophils may be determined by the tissue source of the IL-5 or by a chemotactic/chemokinetic factor derived from the involved tissue that recruits cells already responding to IL-5, or by both.

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1. Centers for Disease Control (1989) *Morbid. Mortal. Wkly. Rep.* **38,** 765–767.
2. Centers for Disease Control (1990) *Morbid. Mortal. Wkly. Rep.* **39,** 89–91.
3. Flannery, M. T., Wallach, P. M., Espinoza, L. R., Dohrenwend, M. P. & Moscinski, L. C. (1990) *Ann. Int. Med.* **112,** 300–301.
4. Varga, J., Peltonen, J., Uitto, J. & Jimenez, S. (1990) *Ann. Int. Med.* **112,** 344–351.
5. Clauw, D. J., Nashel, D. J., Umhau, A. & Katz, P. (1990) *J. Am. Med. Assoc.* **263,** 1502–1506.
6. Hertzman, P. A., Blevins, W. L., Mayer, J., Greenfield, B., Ting, M. & Gleich, G. J. (1990) *N. Engl. J. Med.* **322,** 869–873.
7. Silver, R. M., Heyes, M. P., Maize, J. C., Quearry, B., Vionnet-Fuasset, M. & Sternberg, E. M. (1990) *N. Engl. J. Med.* **322,** 874–881.
8. Winqvist, I., Olofsson, T., Olsson, I., Persson, A.-M. & Hallberg, T. (1982) *Immunology* **47,** 531–539.
9. DeSimone, C., Donelli, G., Meli, D., Rosati, R. F. & Sorice, F. (1982) *Clin. Exp. Immunol.* **48,** 249–255.
10. Prin, L., Capron, M., Tonnel, M.-B., Bletry, O. & Capron, A. (1983) *Int. Arch. Allergy Appl. Immunol.* **72,** 336–346.
11. Kajita, T., Yui, Y., Mita, H., Taniguchi, N., Saito, H., Mishima, T. & Shida, T. (1985) *Int. Arch. Allergy Appl. Immunol.* **78,** 406–410.
12. Fukuda, T., Dunnette, S. L., Reed, C. E., Ackerman, S. J., Peters, M. S. & Gleich, G. J. (1985) *Am. Rev. Respir. Dis.* **132,** 981–985.
13. Owen, W. F., Jr., Rothenberg, M. E., Silberstein, D. S., Gas-

son, J. C., Stevens, R. L., Austen, K. F. & Soberman, R. J. (1987) *J. Exp. Med.* **166,** 129–141.
14. Rothenberg, M. E., Owen, W. F., Silberstein, D. S., Soberman, R. J., Austen, K. F. & Stevens, R. L. (1988) *J. Clin. Invest.* **81,** 1986–1992.
15. Rothenberg, M. E., Petersen, J., Stevens, R. L., Silberstein, D. S., McKenzie, D. T., Austen, K. F. & Owen, W. F., Jr. (1989) *J. Immunol.* **143,** 2311–2316.
16. Owen, W. F., Rothenberg, M. E., Petersen, J., Weller, P. F., Silberstein, D., Sheffer, A. L., Stevens, R. L., Soberman, R. J. & Austen, K. F. (1989) *J. Exp. Med.* **170,** 343–348.
17. Slutsker, L., Hoesly, F. C., Miller, L., Williams, L. P., Watson, J. C. & Fleming, D. W. (1990) *J. Am. Med. Assoc.* **264,** 213–217.
18. McGeer, E. G. & Singh, E. (1984) *Exp. Neurol.* **86,** 410–413.
19. el-Defrawy, S. R., Boegman, R. J., Jhamandas, K. & Beninger, R. J. (1986) *Can. J. Physiol. Pharmacol.* **64,** 369–375.
20. Whetsell, W. O., Jr., & Schwarcz, R. (1989) *Neurosci. Lett.* **97,** 271–275.
21. Garthwaite, G. & Garthwaite, J. (1987) *Neurosci. Lett.* **79,** 35–39.
22. Kim, J. P. & Choi, D. W. (1987) *Neuroscience* **23,** 423–432.
23. Lapin, I. P. (1981) *Epilepsia* **22,** 257–265.
24. Eastman, C. L. & Guilarte, T. R. (1989) *Brain Res.* **28,** 225–231.
25. Pinelli, A., Ossi, C., Colombo, R., Tofanetti, O. & Spazzi, L. (1984) *Neuropharmacology* **23,** 333–337.
26. Sternberg, B. M., Van Woert, M. H., Young, S. N., Magnussen, I., Baker, H., Gauthier, S. & Osterland, C. K. (1980) *N. Engl. J. Med.* **303,** 782–787.
27. Gleich, G. J. & Adolphson, C. R. (1986) *Adv. Immunol.* **39,** 177–253.
28. Sunohara, N., Furukawa, S., Nishio, T., Mukoyama, M. & Satoyoshi, E. (1989) *J. Neurol. Sci.* **92,** 1–7.
29. Fujisawa, T., Abu-Ghazaleh, R., Kita, H., Sanderson, C. J. & Gleich, G. J. (1990) *J. Immunol.* **144,** 642–646.
30. Martin, R. W., Duffy, J., Engel, A. G., Lie, J. T., Bowles, C. A., Moyer, T. P. & Gleich, G. J. (1990) *Ann. Int. Med.* **113,** 124–134.