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ROLE OF SPECIFIC IgE ANTIBODIES IN PEROXIDASE (EPO) RELEASE FROM HUMAN EOSINOPHILS[1]

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After the demonstration of cytophilic IgE immunoglobulins (Ig) on human blood and lung eosinophils, their role in cell activation was studied by eosinophil peroxidase (EPO) assay. Hypodense human eosinophils from filariasis-infected patients were activated by anti-human Ig or various antigens. A selective release of EPO occurred after incubation with anti-human IgE, but not with anti-human IgG. The activation by antigens showed a strict antibody specificity of cytophilic IgE antibodies. The direct involvement of IgE antibodies in activation by the specific antigen was evidenced by inhibition experiments with aggregated human IgE myeloma protein. Circulating IgE antibodies exhibiting the same specificity and able to induce EPO release were detected in the sera from filariasis patients by a passive sensitization assay. Only the hypodense eosinophils were able to release EPO after IgE-dependent activation both in the direct assay and in the passive sensitization test, confirming the functional heterogeneity of human eosinophils. These results suggest that the interaction between IgE antibodies and human eosinophils can play a role both in protective immunity and pathology by releasing active pharmacologic mediators.

The presence of cell-bound IgE has been described on human basophils (1, 2) and rat mast cells (3–5). Similarly, cytophilic IgE have been demonstrated on other $Fc_\epsilon R$-positive cell populations (6–9). The particular function of this second type of $Fc_\epsilon R$ ($Fc_\epsilon R2$) (10) is to participate directly in the cytotoxic capacity of macrophages, eosinophils, and platelets for *Schistosoma mansoni* targets in the presence of IgE antibodies (6, 11, 12). Moreover, eosinophils from highly hypereosinophilic patients showed heterogeneity of this effector function according to the subpopulations of eosinophils used (13, 14). Indeed, eosinophils with lower density, purified on metrizamide gradients (hypodense eosinophils) exhibited an increased IgE-dependent cytotoxic capacity for *S. mansoni* schistosomula (13). More recently, Capron et al. (9) clearly demonstrated the presence of surface IgE on either human blood or tissue eosinophils in several pathological situations, suggesting a role for this IgE-eosinophil interaction in the in vivo effector function of cells.

In previous studies we showed that one granule component, eosinophil peroxidase (EPO),[3] could be released from normal rat eosinophils only after stimulation with anaphylactic Ig (IgG2a and IgE) (15). The in vivo relevance of these findings was established by showing that IgG2a or IgE cytophilic antibodies present on eosinophils from *S. mansoni*-infected rats were also implicated in EPO release (15). Exocytosis of EPO seemed to represent a good marker of eosinophil activation linked to anaphylactic Ig. It was therefore interesting to know whether cytophilic IgE detected on eosinophils from patients with increased circulating IgE levels (9) could also induce specific EPO release after stimulation either with anti-IgE or with specific antigens.

In this paper, a sensitive assay of EPO release was devised to investigate the antibody function of cytophilic IgE and their role in human eosinophil activation. The addition of anti-IgE antibodies or specific antigen to hypodense eosinophils purified from filariasis-infected patients induced specific release of EPO and not eosinophil cationic protein (ECP). Similarly, passive sensitization of hypodense cells by IgE antibodies induced EPO release, suggesting that both cell-bound IgE and circulating IgE with very restricted antibody specificity played a major role in the activation of the hypodense subpopulation of human eosinophils.

MATERIALS AND METHODS

Patients. Two groups of patients without any corticosteroid therapy were selected as eosinophil donors: eight patients with filariasis infections (four with *Loa loa*, three with *Onchocerca volvulus*, and one with *Dipetalonema perstans*) and increased circulating IgE levels (range: 1460 to 4110 IU/ml). Ten patients with hypereosinophilia not linked to parasitic infections and with normal IgE levels served for passive sensitization experiments.

Eosinophil purification. Human leukocytes were collected from heparinized venous blood by dextran sedimentation for 30 min at 37°C. The leukocyte-rich plasma was removed and washed in minimal essential medium (Difco, Detroit, MI) containing 10% heat-inactivated fetal calf serum. The mixed leukocytes were then fractionated by centrifugation upon discontinuous metrizamide gradients as described (16). Highly purified eosinophils (>90%) were usually obtained in the low density layers of metrizamide (20 to 22% metrizamide concentration, $1.115 < d < 1.125$) and are referred to as "hypodense" populations. In the highest density layers (24 to 25% concentration, $1.135 < d < 1.140$) were obtained eosinophils with

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[3] Abbreviations used in this paper: EPO, eosinophil peroxidase; ECP, eosinophil cationic protein; LDH, lactate dehydrogenase; CL, chemiluminescence.

normal density ("normodense") cells (17).

Antibodies and Ig. For the passive sensitization technique, various sera were obtained from the same filariasis patients selected for hypereosinophilia and increased IgE levels. IgE depletion was carried out by solid phase immunoadsorption on anti-IgE-coated Sepharose 4B (Pharmacia, Uppsala, Sweden) (mean levels of IgE before depletion, 2500 IU/ml; after depletion, 10 IU/ml). Human IgE myeloma protein previously aggregated by *bis*-diamino benzidine was a generous gift of Dr H. Spiegelberg (La Jolla, CA). Human normal IgG (Cappel, Cochranville, PA) was aggregated with dimethylsuberimidate as described (11).

Antigens. O. volvulus antigens were prepared from adult worms obtained by dissection of subcutaneous nodules surgically removed from *O. volvulus*-infected patients. The worms were washed twice in 0.1% NaCl and then were desintegrated by six passages through a hydraulic press (X Press Bio; LKB, Bomma, Sweden) at 18,000 pounds per square inch. The crude extract obtained was centrifuged at 15,000 × G for 30 min, and the supernatants were collected and lyophilized. The protein content of the filarial antigen was determined by the Bio-Rad Protein assay (Bio-Rad, München, West Germany). These supernatants were used as such for cell activation. Antigens from *D. viteae*, *S. mansoni*, and *Toxocara canis* adult worms were prepared by the same technique.

L. Loa and *D. perstans* antigens were prepared from microfilariae directly isolated from highly infected patients by apheresis (18). Microfilariae washed extensively in saline-1.7 × 10^{-4} M NaCl were sonicated on ice by using a Pons model 20–200S (Subagnes, France) at 200 W for 15 sec periods, giving a total sonication time of 2 min. The extracts were centrifuged at 18,000 × G for 18 min at 4°C and were dialyzed against phosphate-buffered saline 10^{-2} M-NaCl 9%. The protein content of the resulting supernatants was measured by the Bio-Rad protein assay.

Activation of eosinophils. The capacity of activation after direct stimulation of eosinophils purified from filariasis patients was investigated. Anti-human IgE, anti-human IgG, or specific antigen in various concentrations were added to 0.5 ml samples of eosinophil suspension in Hanks' balanced salt solution (HBSS) (6 × 10^6 eosinophils/ml) and the cells were incubated for 60 min at 37°C. As controls, eosinophils were incubated with medium or unrelated antigens. For displacement experiments, aggregated human IgE or IgG proteins were added to eosinophils and were incubated for 1 hr at 4°C before the addition of specific antigen.

The passive sensitization assay was performed by incubation of eosinophils purified from hypereosinophilic patients with sera from filariasis patients. Eosinophils (6 × 10^6 eosinophils/ml) were sensitized either with total serum or IgE-depleted serum. After 90 min incubation at 4°C, the specific filarial antigen or unrelated antigens were added. In all experiments, eosinophils were incubated for 1 hr at 37°C with occasional shaking. The various protein measurements were thus performed on supernatants recovered by centrifugation at 600 × G for 10 min. Total enzyme contents were measured after lysis of the same cell aliquots by Triton X-100 (0.2% in HBSS).

EPO release assay. The determination of released peroxidase was carried out by a chemiluminescence (CL) assay adapted from Whitehead et al. (19). For the assay, 0.05 ml of supernatants was transferred in plastic tubes containing 0.05 ml H_2O_2 (1.35 mM). Then, 0.05 ml of D-luciferin (160 μM in Tris-HCl 0.01 M, pH 6; Boehringer) and 50 μl of luminol (250 μg/ml in Tris-HCl 0.01 M, pH 6; Serva, Heidelberg, Germany) were added. Each tube was introduced into a photometer (Nucleotimetre 107; Interbio CLV, Paris, France). Light emission was monitored as photocurrent and was expressed as CL units (100 U of CL = 1 mV). At this given pH, no cross-reaction with the peroxidase of neutrophil origin was detected (15).

Lactate dehydrogenase (LDH) assay. The absence of cell lysis was detected by the level of the cytoplasmic marker LDH in the supernatants. LDH was assayed by incubating 0.1 ml of supernatant fluid with 1 ml of sodium phosphate (50 mM, pH 7.5) and 0.1 ml of nicotinamide-adenine dinucleotidase hydrogen (4 mM). The reaction was then started by the addition of 0.1 ml of 10 mM sodium pyruvate. Total LDH was measured in Triton-lysed eosinophils. LDH activity was estimated by the disappearance of absorption at 340 nm.

ECP assay. Human ECP was measured by radioimmunoassay by using a monoclonal anti-ECP antibody. Briefly, 50 μl of supernatants were added to polyvinylchloride microtiter plates coated with 50 μl of anti-ECP antibody (5 μg/ml). After incubation 1 hr at 37°C, the amount of ECP bound was assessed by adding 50 μl of ^{125}I-labeled anti-ECP antibody. The results were expressed as micrograms per milliliter by using a standard curve constructed with purified ECP.[4]

[4] **Po-Chun, T., M. Capron, D. M. Bakes, J. Barkans, and C. J. F. Spry** 1985. Monoclonal antibodies to human eosinophil plasma membrane antigens enhance the secretion of eosinophil cationic protein. *Submitted for publication.*

Analysis of data and statistical study. The results were expressed as mean ± SD and were analyzed by using Student's *t*-test.

RESULTS

EPO release after stimulation with anti-human-IgE. To investigate the role of cytophilic IgE in eosinophil activation, the release of peroxidase exocytosed after stimulation with anti-human Ig was measured by a sensitive CL technique. Hypodense eosinophils from filariasis patients directly incubated with anti-human IgE antibodies released significant amounts of EPO up to 5% of the total content (Table I). Under the same conditions, the addition of anti-human IgG antibodies was inefficient. In contrast, eosinophils recovered from high density layers (normodense cells) showed no significant release of EPO in the presence of anti-IgE, whereas with anti-IgG, low but significant levels of EPO were released (Table I).

Activation by specific antigens. To demonstrate the antibody specificity of these cytophilic IgE, EPO release was measured after incubation of eosinophils from filariasis patients with various antigens. As shown in Figure 1, which illustrates four representative experiments, the incubation of "hypodense" cells with the antigen related

TABLE I

Release of EPO from different populations of eosinophils by various anti-human Ig antibodies

Eosinophils incubated with[a]	EPO (CL units)[b]	
	Hypodense	Normodense
Anti-IgE	7,501 ± 2,323[c] (7)	691 ± 217 (3)
Anti-IgG	1,523 ± 860 (5)	1,433 ± 107 (2)[d]
HBSS	2,351 ± 753 (7)	644 ± 202 (3)

[a] Purified eosinophils (>90%, 6 × 10^6 cells/ml) were incubated with various anti-human Ig antibodies (final dilution 1/10) for 1 hr at 37°C.

[b] EPO activity in supernatants was tested in the presence of H_2O_2, luminol, and luciferin. The results are expressed as CL units (mean ± SD). Numbers in parentheses are the numbers of experiments.

[c] Significantly higher than controls ($p < 0.001$).

[d] Significantly higher than controls ($p < 0.05$).

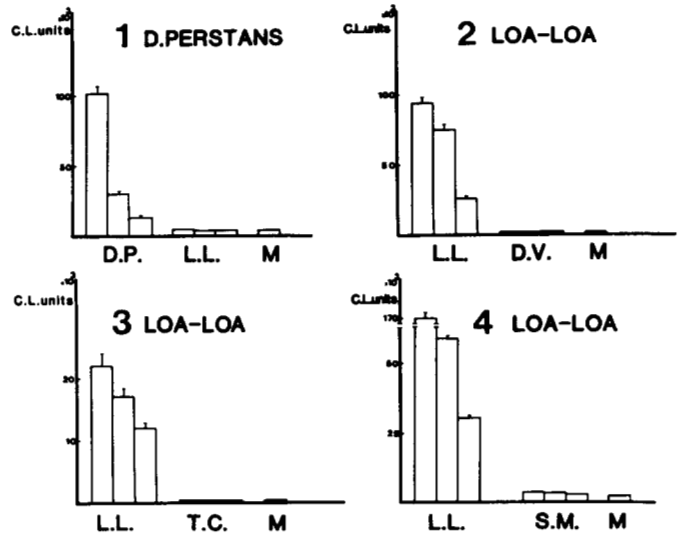

Figure 1. Induction of EPO release by eosinophils purified from filariasis patients. *1, D. perstans; 2, 3,* and *4, L. Loa* infection. Hypodense eosinophils were incubated with *D. perstans* (*D.P.*), *L. loa* (*L.L.*), *D. viteae* (*D.V.*), *T canis* (*T.C.*), or *S. mansoni* (*S.M.*) antigen (80, 40, or 20 μg/6 × 10^6 cells respectively) or medium (*M*) for 1 hr at 37°C. EPO releases in the supernatants were measured in the presence of H_2O_2, luminol, and luciferin. The results are expressed in CL units (mean of duplicate experiments ± SD).

to the patient infection induced high extracellular release of EPO (between 10 and 20% of the total content). Moreover, EPO release was dependent upon the concentration of the specific antigen added. In contrast, no significant EPO exocytosis was detected after incubation with any of the unrelated antigens (Fig. 1).

Comparison of the activation of "hypodense" and "normodense" eosinophils. To compare the capacity of activation of the various eosinophil populations, the induction of EPO release from hypodense or normodense eosinophils in the presence of specific antigen or anti-Ig was performed. The results presented in Table II revealed that only hypodense eosinophils were able to release EPO in the presence of specific antigen (*O. volvulus*) or anti-human IgE. In contrast, the addition of antigen or anti-IgE did not induce EPO release from normodense cells. Similarly to results presented in Table I, supernatants obtained after the addition of anti-human IgG antibodies showed significant peroxidase activity only in the case of normodense cells. It is important to note that after treatment with Triton X-100, normodense eosinophils had higher levels of intracellular EPO than the hypodense populations.

Kinetics of EPO and LDH release. The time course of EPO and LDH release during the activation of hypodense eosinophils was studied. The comparison of EPO release by human anti-IgE or specific antigen showed that EPO activity was detectable after 15 min of incubation and reached a plateau at 90 min (Fig. 2). The rate of EPO release was greater in the presence of specific antigen than in the presence of anti-IgE. No leakage of LDH from the cells was obtained under the same conditions of activation.

Direct evidence for the role of cytophilic IgE by displacement experiments. For a clearcut demonstration of the participation of antigen-specific IgE in the direct activation of hypodense eosinophils measured by EPO exocytosis, inhibition experiments of EPO release after preincubation with aggregated human Ig were performed. In two patients with *L. loa* infection, a first step of incubation (60 min at +4°C) of the cells with aggregated IgE induced an 80% reduction of EPO release after the addition of specific antigen (Fig. 3). In contrast, preincubation with a 10-fold excess of aggregated IgG induced no change in the extracellular release of EPO.

ECP assay. Because hypodense eosinophils with cell-bound IgE appear to release EPO from granules in the presence of specific antigen or anti-human IgE, it was of interest to see whether another granule component, ECP, could also be detected in the supernatants of eosinophils treated in the same conditions. The results of two representative experiments shown in Table III revealed that ECP was not released neither after incubation with anti-IgE nor with the specific antigen, whereas significant amounts of ECP were released after anti-IgG stimulation.

Passive sensitization of eosinophils by serum of filariasis patients. With the above results suggesting that exocytosis of EPO was linked to the presence of cytophilic

TABLE II

Comparison of EPO release from hypodense and normodense eosinophils purified from patients with O. volvulus

Eosinophils Incubated with[a]	EPO (CL units)[b]	
	Hypodense	Normodense
HBSS	861 ± 2	902 ± 25
T. canis[c]	618 ± 115	406 ± 78
S. mansoni[c]	706 ± 140	647 ± 64
O. volvulus[c]	5,670 ± 112[d]	1,107 ± 200
Anti-human IgE[e]	3,417 ± 279[d]	1,225 ± 106
Anti-human IgG[e]	888 ± 213	1,541 ± 73[f]
Total EPO[g]	97,065 ± 1,945	127,505 ± 12,495

[a] Purified eosinophils were incubated for 1 hr at 37°C.
[b] Results are expressed in units of CL (mean ± SD).
[c] Purified antigen (30 μg) was added per 6 × 10^6 cells/ml.
[d] Significantly higher than controls ($p < 0.01$).
[e] Anti-human Ig were used at 1/10 final dilution.
[f] Significantly higher than controls ($p < 0.05$).
[g] Total intracellular EPO were obtained after lysis of the same quantity of eosinophils with Triton X-100 (0.2% final dilution).

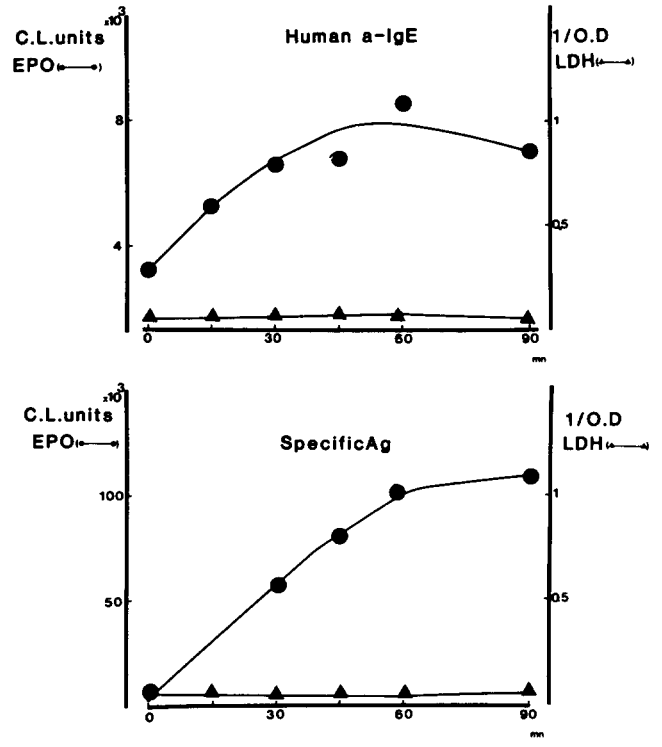

Figure 2. Kinetics of EPO (●—●) and LDH(▲—▲) released by eosinophils from filariasis patients. Hypodense eosinophils were incubated with anti-human IgE (1/10 final dilution) or with specific antigen (80 μg/6 × 10^6 cells) for 1 hr at 37°C. The results of EPO release are expressed as CL units and LDH as 1/O.D.

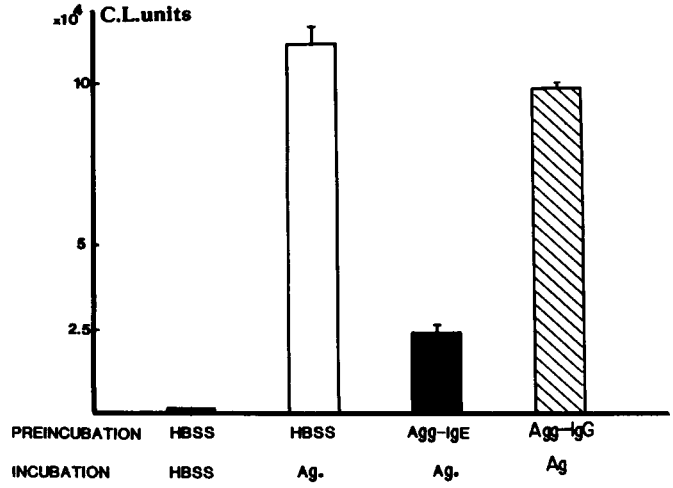

Figure 3. Displacement of cytophilic IgE bound in vivo to eosinophils from filariasis patients. Hypodense eosinophils were preincubated with medium (HBSS), aggregated IgE (30 μg/6 × 10^6 cells), or aggregated IgG (300 μg/6 × 10^6 cells) for 1 hr at 4°C and then with medium or specific antigen (80 μg/6 × 10^6 cells) for 1 hr at 37°C. The results of EPO release are expressed as CL units (mean of two duplicate experiments ± SD).

TABLE III

Comparative release of EPO and ECP by hypodense eosinophils

Incubation for 60 min[a] at 37°C with	EPO		ECP	
	CL units	% of total[b]	μg/ml[b]	% of total[b]
Experiment 1				
HBSS	13,208 ± 1,478	2.2	0.42 ± 0.01	2
Specific antigen (*L. loa*)[c]	55,651 ± 2,103	11[d]	0.59 ± 0.00	2.8
Anti-IgE[e]	31,615 ± 2,993	6.6[d]	0.69 ± 0.08	3.3
Anti-IgG[e]	18,890 ± 390	3.6	2.13 ± 0.29	10.3[d]
Triton[f]	523,360 ± 5,113	—	20.16 ± 0.16	—
Experiment 2				
HBSS	2,997 ± 436	1.9	0.32 ± 0.01	0.6
Specific antigen (*L. loa*)[c]	20,163 ± 112	13.4[d]	0.52 ± 0.01	1.1
Anti-IgE[e]	7,835 ± 257	5.2[d]	0.47 ± 0.01	1.0
Anti-IgG[e]	2,729 ± 430	1.8	1.23 ± 0.00	2.6[d]
Triton[f]	150,386 ± 11,923	—	46.53 ± 2.97	—

[a] Hypodense eosinophils were recovered from the 20 to 22% metrizamide layers.
[b] Percent of total = CL units or μg/ml obtained in the presence or absence of stimulus + CL units or μg/ml obtained after lysis of cells with Triton X-100.
[c] Specific antigen (*L. loa*) was used at 40 μg for 6×10^6 cells/ml.
[d] Significantly higher than control ($p < 0.01$).
[e] Anti-human Ig antibodies were used at 1/10 final dilution.
[f] Total intracellular EPO and ECP were obtained after lysis of the same quantity of eosinophils with Triton X-100 (0.2% final dilution).

IgE on hypodense cells, it was interesting to investigate whether circulating IgE antibodies were able to passively sensitize eosinophils. To address this point, hypodense eosinophils purified from hypereosinophilic patients were sensitized with the sera from filariasis patients and then were activated by the addition of the specific antigen. As shown in Table IV, after 90 min of incubation of hypodense cells with the serum of filariasis patients, eosinophils were able to release EPO after a subsequent 60 min incubation at 37°C with the specific antigen. The involvement of IgE antibodies in this mechanism of passive sensitization was demonstrated by the decrease in EPO exocytosis when the sera have been adsorbed on an anti-IgE column. Similarly to the direct assay, only the hypodense eosinophils can be passively sensitized by IgE containing sera to release EPO content (Table V).

DISCUSSION

Previous studies have demonstrated that rat eosinophils were able to release significant levels of EPO after passive sensitization with anaphylactic Ig (IgG2a or IgE) (15). Moreover, the incubation of eosinophils from *S. mansoni*-infected rats with the specific antigen confirmed that cytophilic antibodies with anaphylactic properties were present at certain periods of infection, and were directly involved in cell activation as measured by exocytosis of one granule component, EPO. In the present report, we have extended this finding to study the functional role of cytophilic IgE previously described on human eosinophils by flow microfluorometry (14). The measurement of EPO release was preferred for several reasons. First was the availability of a very sensitive assay by a CL procedure more adapted to human cells than the enzymatic method used for rat eosinophils. Second was the possibility of working at a given pH to detect only EPO and not myeloperoxidase from the few contaminating neutrophils. Third was the fact that EPO is not only a marker of exocytosis but also a cytolytic molecule (20). Finally, the high levels of rat EPO release mediated by anaphylactic Ig led us to investigate the interaction of IgE with human eosinophils.

The release of EPO from human eosinophils after stimulation with anti-IgE antibodies confirmed the existence of cytophilic IgE and their involvement in cell activation. Such results suggest therefore that $Fc_\epsilon R2$ present on human eosinophils have sufficient affinity to bind IgE in vivo and can play a role in the effector function. No EPO was released after interaction with anti-IgG. The fact

TABLE IV

Passive sensitization of hypodense cells by serum from filariasis patients

First Incubation[a] (90 min, 4°C)	Second Incubation[b]	CL Units[c]	p Value
Experiment 1			
HBSS	HBSS	1,293 ± 10	—
HBSS	30 μg *O. volvulus* antigen	2,196 ± 53	NS[e]
O. volvulus serum[d]	HBSS	1,991 ± 40	NS[e]
O. volvulus serum[d]	10 μg *O. volvulus* antigen	3,151 ± 98	$p < 0.01$
O. volvulus serum[d]	30 μg *O. volvulus* antigen	3,886 ± 146	$p < 0.01$
O. volvulus serum[d]	90 μg *O. volvulus* antigen	6,205 ± 406	$p < 0.001$
IgE-depleted *O. volvulus* serum[d]	90 μg *O. volvulus* antigen	1,223 ± 64	NS[e]
Experiment 2			
HBSS	HBSS	1,228 ± 113	—
HBSS	40 μg *L. loa* antigen	1,070 ± 28	NS[e]
L. loa serum[d]	HBSS	1,120 ± 83	NS[e]
L. loa serum[d]	40 μg *L. loa* antigen	3,545 ± 148	$p < 0.01$
IgE-depleted *L. loa* serum[d]	40 μg *L. loa* antigen	1,500 ± 99	NS[e]

[a] Hypodense eosinophils were used at 6×10^6 cells/ml.
[b] Cells were incubated without washing, with HBSS, or with specific antigens for 1 hr at 37°C.
[c] Results are expressed as units of CL (mean ± SD).
[d] Total sera recovered from filariasis patients or IgE-depleted sera were used at 1/10 final dilution.
[e] NS: not significant when compared with the control.

TABLE V

Comparison of EPO release from hypodense and normodense eosinophils in the passive sensitization test

First Incubation (90 min, 4°C)[a]	Second Incubation (60 min, 37°C)	CL Units[b]	
		Hypodense cells	Normodense cells
HBSS	HBSS	1,692 ± 154	408 ± 26
HBSS	30 μg *O. volvulus* antigen	1,794 ± 121	ND[c]
O. volvulus serum[d]	HBSS	2,302 ± 51	492 ± 30
O. volvulus serum[d]	30 μg *O. volvulus* antigen	6,740 ± 327[e]	288 ± 52

[a] Purified eosinophils were used at 6×10^6 cells/ml.
[b] Results are expressed in units of CL (mean ± S.D.).
[c] ND = not done.
[d] Total sera recovered from patients with *O. volvulus* infection were used at 1/10 final dilution.
[e] Significantly higher from controls ($p < 0.01$).

that EPO was released by a secretory process and not as the consequence of cell death was shown by the kinetic experiments of EPO and LDH release. EPO was released as soon as 15 min after the addition of the stimulus, and the levels increased during the subsequent 90 min, whereas no LDH could be detected in the supernatants. These results are in agreement with those previously reported on EPO release from human eosinophils activated by calcium ionophore (21) or by the human IgE-anti-IgE complex (22).

Studies on the antibody specificity of such cytophilic IgE could be performed on filariasis patients because in filarial infections, hypereosinophilia and increased IgE levels were often associated; in addition, there was the recent possibility to use purified specific antigens of human infections (18). Eosinophils purified from filariasis patients and incubated with various antigens were able to release significant amounts of EPO (from 5 to 20% of the total content) only after incubation with the specific antigen. The absence of EPO release from eosinophils incubated in the presence of unrelated antigens, even when they are very close antigenically to the specific antigen, such as *L. loa* and *D. perstans* for instance, showed the strict antibody specificity of IgE antibodies. These observations not only demonstrate the activation of cell-bound IgE by the specific antigen, but also suggest the presence of species-specific determinants on various filarial worms (23). This has never been reported in the case of IgG antibodies, which showed large cross-reactivity (24). The very strict antibody specificity of cytophilic IgE might be due to the binding of IgE in a given molecular form, probably aggregated with antigens forming immune complexes with a higher affinity for $Fc_\epsilon R2$ (25).

The levels of EPO released after incubation with the specific antigen were always superior to those obtained after anti-IgE stimulation. The major involvement of IgE antibodies in the case of addition of the specific antigen was evidenced by displacement experiments. Although preincubation of eosinophils with an excess of aggregated IgG induced no change in EPO release, the preincubation with aggregated IgE significantly inhibited the extracellular release of EPO after antigenic stimulation, suggesting that at least 80% of the release was due to IgE. There is no clear explanation for the lower level of EPO released by anti-human IgE. Several hypotheses might be proposed such as the low avidity of the various anti-human IgE used, or the lack of accessibility of cell bound IgE to the anti-Fc (IgE) antibodies.

Our experiments clearly confirmed the heterogeneity of human eosinophil populations, because only cells with low density (hypodense) were able to release EPO, both in the case of the direct assay or after passive sensitization. In this respect, it is important to notice that the higher levels of EPO released from hypodense cells did not seem related to the amounts of intracellular peroxidase, because much more peroxidase was present in the normodense populations. Therefore, our results suggest that the decrease in EPO levels present in hypodense eosinophils could be due to a previous in vivo release of EPO, probably induced by an IgE stimulus. This eosinophil heterogeneity measured by a very sensitive functional assay (EPO release) is in agreement with previous findings showing that hypodense human eosinophils were more cytotoxic than normodense cells against *S. mansoni* targets in the presence of IgE antibodies (11). Moreover, recent observations have reported the role of peroxidase-dependent oxydative metabolism in such an IgE-dependent killing mechanism (26).

In respect to the numerous works concerning the release of major basic protein (MBP) and ECP, it was interesting to investigate the selectivity of EPO release by comparison with ECP release. In our experimental conditions no correlation was found between EPO and ECP release, because after addition of anti-IgE or antigen, no ECP could be detected. In contrast, ECP can be detected after the addition of human IgG, whereas no EPO release was induced. These findings might be related either to the heterogeneity of eosinophil subpopulations (hypodense/normodense) in response to a given stimulus or to a variability in the response of a given eosinophil population to different stimuli (IgE/IgG for instance). Indeed, MBP or ECP was clearly shown to be released after interaction of eosinophils with IgG or complement (27, 28). Similarly, the participation of both ECP and MBP in the IgG-mediated killing by normodense eosinophils has been reported (27, 29).

The participation of IgG antibodies was clearly ruled out in passive sensitization experiments that showed that after IgE depletion the sera from infected patients was unable to induce EPO release. However, these results have to be confirmed by using IgG antibodies of different specificities and also by varying the experimental conditions. Indeed, EPO release was measured after a maximum of 90 min of incubation, whereas it was shown in the case of rat macrophages that the IgG-anti-IgG triggering required at least 6 hr of contact to reach the levels of B-glucuronidase obtained after 30 min in the case of IgE-anti-IgE activation (30). Investigations on the release of EPO, ECP, and MBP after IgG or IgE-dependent stimulation of the various eosinophil populations will certainly provide useful information on the in vitro and in vivo effector functions of human eosinophils.

These studies confirmed the presence of cytophilic IgE antibodies and demonstrated that IgE-bearing eosinophils could be activated by the specific antigen to degranulate and release peroxidase. If the cytolytic role of EPO for parasites, bacteria, or tumor cells (20, 31, 32) has been clearly evidenced, giving to eosinophils a major function in protection, it is also possible that IgE-peroxidase-dependent mechanisms can play a role in pathology. First, specific IgE have been implicated in histamine release from blood leukocytes obtained from filariasis patients (5). Higher levels of histamine were obtained from patients with tropical eosinophilia than with non-tropical eosinophilia. Such findings could be due to the EPO released by contaminating eosinophils, because EPO supplemented with H_2O_2 and halide is able to induce mast cell degranulation (33). More recently, EPO was shown to be directly involved in cytolytic processes against lung cells (34, 35), pointing out the potential role of this IgE-dependent EPO release in lung pathology.

In conclusion, our results confirm that IgE-bearing eosinophils can be directly activated by specific antigens to release active pharmacological mediators. Among these mediators, peroxidase seems to play a major role in the dual effector function of eosinophils both in protection, by killing parasite larvae, and in diseases associated with increased IgE production and hypereosinophilia.

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