Clin. exp. Immunol. (1986) **63,** 249–257.

Eosinophilic lung disease: immunological studies of blood and alveolar eosinophils

L. PRIN, M. CAPRON, P. GOSSET, B. WALLAERT, J. P. KUSNIERZ, O. BLETRY, A. B. TONNEL & A. CAPRON *Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U 167 – CNRS 624, Institut Pasteur, Lille, France*

(Accepted for publication 24 July 1985)

SUMMARY

Five patients with eosinophilic lung diseases and blood hypereosinophilia (PIE syndrome) were investigated clinically and by bronchoalveolar lavage (BAL). Comparative studies on blood and alveolar eosinophils were carried out after purification and selection of eosinophil subpopulations according to their density. A predominant 'hypodense' alveolar eosinophil population was found in BAL fluids of active chronic eosinophilic pneumonia (CEP). In addition, supernatants of alveolar macrophages obtained from CEP are able to enhance spontaneously the generation of eosinophil oxygen metabolites. Such eosinophil stimulation emphasizes a probable tissue cell cooperation. In addition, BAL permitted the study of membrane immunological markers on eosinophilic inflammatory cells endowed with migratory properties. An increase in eosinophils carrying surface IgE was demonstrated in alveolar cells from PIE Syndrome particularly with hypodense eosinophils from CEP patients. Although no specific stimulus is known at the present time, this work underlines the potential implication of IgE-mediated hypersensitivity processes in the pathogenesis of eosinophilic lung diseases.

Keywords eosinophilic lung disease bronchoalveolar lavage cytophilic IgE eosinophil heterogeneity

INTRODUCTION

Previous work has underlined the toxic potential of human eosinophils against helminths (Butterworth *et al.*, 1975) or mammalian target cells (Gleich *et al.*, 1979; Jong & Klebanoff, 1980). This toxicity mediated by eosinophils may explain some of the detrimental effects and tissue damage observed in prolonged hypereosinophilia in humans, i.e. cardiomyopathy (Tai *et al.*, 1982; Spry, Tai & Davies, 1983) or neuropathy (Durack, Sumi & Klebanoff, 1979; Fredens, Dahl & Venge, 1982). Eosinophil cationic peptides (Gleich *et al.*, 1979; Fredens, Dahl & Venge, 1982), eosinophil-derived neurotoxin (Durack *et al.*, 1981) and/or the peroxidase system (Jong, Mahmoud & Klebanoff, 1981) might represent some of the molecules implicated in these effector mechanisms. Eosinophilic bronchopathy has also been described (Frigas, Loegering & Gleich, 1980). Although the precise role of eosinophils in pulmonary infiltrates remains unclear, eosinophilic pneumonia has been associated with acute and chronic alterations in histological findings and pulmonary functional tests (Carrington *et al.*, 1969; Morrissey *et al.*, 1975; Fox & Seed, 1980).

Five cases of eosinophilic lung diseases with blood hypereosinophilia (PIE syndrome) were

Correspondence: Dr Lionel Prin, C.I.B.P., Institut Pasteur, 15, rue Camille Guérin, 59019 Lille Cédex, France.

studied. Blood eosinophils were compared to tissue eosinophils obtained by bronchoalveolar lavage (BAL). Separate eosinophil subpopulations were selected by a density difference ('normodense' and 'hypodense' eosinophils) which has previously allowed us to characterize immunological (Prin *et al.*, 1983) and metabolic (Prin *et al.*, 1984) eosinophil heterogeneity. A predominant 'hypodense' alveolar eosinophil population was found in chronic eosinophilic pneumonia (CEP) with a particular status of *in vivo* activation possibly induced by alveolar macrophages.

The presence of Fcε receptors (Capron *et al.*, 1981; 1984) and cytophilic IgE (Capron *et al.*, 1985) on the surface of eosinophils has been demonstrated. In this study, BAL allowed the evaluation of these immunological markers on lung eosinophil membranes, showing an increase of surface IgE on hypodense alveolar eosinophils obtained from CEP. The pathogenesis of IgE-mediated processes in eosinophilic lung diseases is discussed.

MATERIALS AND METHODS

Source of blood and alveolar eosinophils: case reports. Five patients, all non-smokers, with pulmonary eosinophilia were studied during the active phase of lung disease: three cases of CEP, one case of polyarteritis nodosa (PAN) and one case of amiodarone associated drug hypersensitivity (DH) (Table 1).

The three CEP patients presented most of the usual signs of this disease, i.e., weight loss, fever, night sweats, dry cough, severe dyspnea without wheezes but with widespread inspiratory crackles. In cases 1 and 2, chest X-rays showed homogeneous dense infiltrates of the two upper lobes in the form of 'helmet crests'. In case 3, diffuse infiltrates predominated in both upper lobes and in the right Fowler segment. All patients were free of allergic, drug-induced or systemic diseases, and tests for fungal and parasitic infections were negative. Lung function tests were altered particularly in two cases with a restrictive pattern, a severe hypoxemia and gas transfer defect. Because of hypoxia and the potential risk of pneumothorax, BAL was preferred to transbronchial or open lung biopsy at this initial stage.

In the PAN case, diffuse arthralgia, severe asthma and peripheral neuropathy were associated with a typical necrotizing vasculitis at muscular biopsy.

In the last clinical case, despite the unusual amiodarone-associated eosinophilia, the lack of evidence for the other known causes of eosinophilia and the favorable course after discontinuation of the drug led to the diagnosis of drug-induced eosinophilic pneumopathy.

In all tested cases, BAL was performed in the areas of radiologic infiltrates during active clinical

Table 1. Clinical, functional and biological data in five patients with eosinophil pulmonary infiltrates

	Chronic eosinophilic pneumonia			Polyarteritis nodosa	Drug hypersensitivity pneumonitis
	Case 1	Case 2	Case 3		
Age (years)	40	34	37	46	56
Sex	F	F	F	F	M
Dyspnea	severe	severe	mild	severe	severe
Chest radiography	'Helmet crest'		infiltrates	Diffuse infiltrates	Basal infiltrates
Pulmonary function	restrictive	restrictive	normal	obstructive	restrictive
PaO_2 (mmHg)	57	55	80	60	65
Erythrocyte sedimentation rate (ESR)	112	118	40	50	48
Blood eosinophil count (%)	25	23	14	27	66
nb/mm³	3250	4070	1160	3830	9300
Serum IgE (IU/ml) (normal value-PRIST < 120)	612	531	543	679	405

disease. The patients did not receive any therapy at the time of the study and were not known to have received any therapy in the preceding months.

Ten control subjects without blood hypereosinophilia were included in this study: one case of CEP in remission, one case of chronic sarcoidosis, one case of infectious pneumonia and seven healthy volunteers. All subjects were informed about the procedure and consented to be investigated.

Cell preparations. Blood cells and alveolar cells were prepared as previously detailed (Prin *et al.*, 1983, 1984). Briefly, venous blood was collected in heparinized tubes. Blood leucocytes were obtained by dextran sedimentation of erythrocytes and washed in minimum essential medium (MEM) (Difco, Detroit, MI, USA). Alveolar cells were obtained using BAL which was performed under fiberoptic bronchoscopy (BFB_3, Olympus, Scop Medicine, Paris, France) by injection of 50 ml aliquots of 250 ml physiological saline in a subsegmental bronchus. The first 50 ml corresponding to bronchial lavage were recovered and discarded. The following four injections, considered as the alveolar lavage, were recovered and centrifuged at 200 ***g*** for 10 min at 4°C. Blood and alveolar total cell counts were carried out. Cell identification was performed using cytocentrifuge preparations stained by Giemsa (RAL 555, Société Chimique Pointet Girard, Clichy, France). Comparative total cell counts and differential cell counts (%) in BAL fluid from PIE syndromes or controls are given in Table 2.

Density gradient separation of blood and alveolar eosinophils in PIE syndrome. Blood and alveolar cells were suspended at $0{\cdot}5 \times 10^8$ cells/ml in MEM containing 10% heat-inactivated fetal calf-serum (MEM/FCS) and were layered on discontinuous metrizamide gradients (Nyegaard, Oslo, Norway) as previously described (Prin *et al.*, 1983). The cell fractions were then collected from each gradient and interface and washed in MEM. The percent purity and the morphology of the eosinophils were estimated by using cytocentrifuge preparations stained with Giemsa. Normal granular eosinophils which are collected in 24–25% metrizamide solutions (density 1·135 to 1·140 g/ml) are referred to as 'normodense' eosinophils. Eosinophils which sediment in the lightest density gradients (in 18 to 23% metrizamide solutions; density 1·105 to 1·125 g/ml) are referred to as 'hypodense' eosinophils (Prin *et al.*, 1983; 1984). No contaminating basophils or mast cells were identified in these purified blood or alveolar eosinophil preparations. Viability estimated by the trypan blue dye exclusion test was >90% for all separated subpopulations.

Activation of cells by alveolar macrophage (AM) supernatants.

(a) *Macrophage isolation.* After centrifugation, the pellet of alveolar cells was suspended in MEM/FCS supplemented with 100 iu penicillin/ml and 50 μg streptomycin/ml (Specia, Paris, France). The cells ($2{\cdot}5 \times 10^6$) were then plated in 35 mm diam plastic petri dishes (Nunc, Roskilde, Denmark) and allowed to adhere for 2 h at 37°C in a 5% CO_2 incubator. After elimination of non-

Table 2. Cell populations in bronchoalveolar lavage

	PIE syndrome					Controls				
	CEP (active phase)								Healthy subjects	
	1	2	3	PAN	DH	CEP (remission)	Chronic sarcoidosis	Infectious pneumonia	non-smokers (3)	smokers (4)
Total cell count ($\times 10^6$ cells)	190	100	72	210	160	18	39	14·5	12·1 ± 3·5	55·9 ± 21·5
Differential cell count (%)										
Eosinophils	61	32	28	75	80	8	9	0	0·8 ± 0·2	0·75 ± 0·8
Macrophages	23	52	65	22	19	48	55	53	86·6 ± 2·9	96·7 ± 1·9
Lymphocytes	8	8	5	2	1	40	36	30	10·6 ± 2·5	2·2 ± 1·3
Neutrophils	8	8	2	1	0	4	0	17	1·5 ± 0·4	0·25 ± 0·4

adherent cells, the remaining cells (98% viable by trypan blue dye exclusion) were esterase positive (>97% AM).

(b) *Preparation of AM supernatants.* After a previous step of adherence, 3×10^6 AM were incubated in 2 ml of MEM for 2 or 3 h at 37°C in a 5% CO_2 in air atmosphere without any stimulation. Supernatants were collected and frozen at −20°C and their activity on purified blood normodense eosinophil suspensions was then evaluated by chemiluminescence (CL).

Chemiluminescence (CL) assay. CL was assessed as previously (Prin *et al.*, 1984). Briefly, eosinophil-rich suspensions (blood 'normodense' eosinophils > 85% purity from different healthy volunteers) were diluted in Hank's balanced salt solution (HBSS) ($1{\cdot}5 \times 10^6$ cells/ml) and kept at 4°C before testing. One hundred microlitres of cell suspensions were transferred into a counting tube, previously filled with either 100 μl of MEM (basal state) or AM supernatants at different final dilutions of 1/2, 1/4 or 1/10. Fifty microlitres of dark-adapted luminol solution (Sigma Chemical Co., Poole, Dorset, UK) diluted 1/10 in HBSS were added (luminol dissolved in dimethylsulphoxide at a concentration of 2 mg/ml). After 18 min or 60 min incubation at 37°C, integration of light emission was measured during 30 s (ct/min). In an automatic photometer (Nucleotimetre 107, Interbio CLV, Paris, France) (Descamp-Latscha *et al.*, 1982).

Fluorescence flow microfluorometry (FMF) analysis.

(a) *Reagents.* Immunoglobulins: human IgE myeloma protein (BL) was purchased from Bernett Labs (Buena Park, CA, USA). Purified human IgG was prepared from normal human serum by ion exchange chromatography.

Antisera: fluorescein isothiocyanate (FITC)-labelled ($F(ab')_2$ fragments anti-human IgE sheep antibodies and antihuman IgG goat antibodies were obtained from Cappel Laboratories Cochranville, PA, USA).

(b) *Cell-labelling.* Purified leucocytes (5×10^6 cells/ml in Hank's medium) were first incubated at 4°C either for 90 min (with medium or 100 μg IgE) or for 60 min with IgG (2 mg). After one wash, the respective FITC-labelled antibodies were added for 30 min at 4°C at a final dilution of 1/10 (for anti-IgE) or 1/20 (for anti-IgG). After two washes, the cells were fixed with paraformaldehyde (U.C.B., Belgium, 1% final concentration) as previously shown (Capron *et al.*, 1985).

(c) *FMF analysis.* The choice of both the reagents and optimal test conditions were selected on the basis of previous work in which different experimental variables were assessed (Capron *et al.*, 1985). Briefly, the expression of cell surface markers was studied with an Ortho flowmicrofluorometer (Cytofluorograf 50-H, Ortho Diagnostic systems, Westwood, MA, USA) equipped with argon-ion laser and helium-neon laser which gives multiparametric data in 512 channels (light scatter, axial light loss used to discriminate eosinophil population and relative fluorescence intensity to evaluate the percentage of fluorescent cells). The results are expressed as percentage of cells with fluorescence intensity above the threshold as previously detailed (Capron *et al.*, 1985) (i.e, percentage of 'positive' cells in the peak or percentage of fluorescent cells) (Hoffman & Hausen, 1981).

RESULTS

BAL fluid cell recovery in PIE Syndrome

The total cell count in BAL fluid from PIE Syndrome showed a very large recovery of alveolar cells ($146{\cdot}4 \pm 26{\cdot}3 \times 10^6$ cells), significantly higher than usually observed in non-smokers. The eosinophil count was also elevated ($90{\cdot}8 \pm 27{\cdot}4 \times 10^6$) in the acute stage of eosinophilic pneumonia. Even though the percentage of alveolar macrophages (AM) reached only $36{\cdot}2 \pm 9{\cdot}4\%$ of the total fluid cells, their absolute number was higher in CEP than in controls. Neutrophils (level superior to 3%) were also identified in 2 cases of CEP (n°1 and n°2) (Table 2).

Using a cell purification procedure through density gradients, we selected 'normodense' and 'hypodense' eosinophil subpopulations (Fig. 1). A large proportion of the blood eosinophils were 'normodense' in controls ($77{\cdot}5 \pm 6{\cdot}3\%$) and in patients with PIE Syndrome ($64{\cdot}0 \pm 1{\cdot}6\%$). In contrast, 'hypodense' eosinophils were predominant in BAL fluid from CEP ($86{\cdot}3 \pm 11{\cdot}2\%$) while in

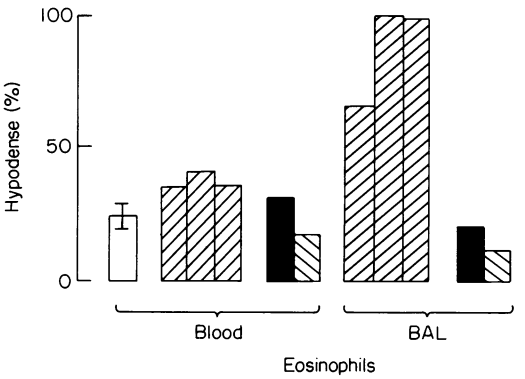

Fig. 1. Distribution of blood and alveolar eosinophils after separation on discontinuous gradients of metrizamide: Percentage of hypodense eosinophils in patients with PIE syndrome and in control subjects without blood hypereosinophilia. (□) Controls; (■) CEP; (■) PAN, (▨) Drug-hypersensitivity.

the other two PIE Syndromes, the eosinophil distribution was similar in blood and in BAL fluid (15 to 30% 'hypodense' cells).

CL stimulation of eosinophils by AM supernatants

Highly purified AM (>97%) from the three CEP patients and from ten controls were cultured for 3 h without any previous stimulation. Among the controls, AM from BAL fluids with quite different total and differential cell counts were included (Table 2). Control AM supernatants did not modify the base CL values of normodense blood eosinophils derived from three different donors. In contrast, AM supernatants from the three CEP patients released factors which are able to enhance significantly the luminol dependent CL of these eosinophils ($P < 0{\cdot}001$, compared to the base CL values) (Table 3). No significant difference in eosinophil CL stimulation was observed with active AM supernatants derived from 2 or 3 h cell cultures. A dose-dependent effect of these active AM factors was noted. It was associated with a maximal eosinophil CL stimulation after 18 min incubation (Table 4).

Table 3. CL modulation of eosinophils by AM supernatants

AM supernatants† obtained from	Chemiluminescence (CL; in ct/min)* Basal state (mean ± s.e.)	after addition of AM supernatants (mean ± s.e.)
CEP (three patients)	3402 ± 201	9835 ± 837
Control subjects‡ (ten controls)	3807 ± 674	2719 ± 479

* CL modulation after 18 min incubation at 37°C (each experiment was done in triplicate on normodense blood eosinophils purified from three different donors).

† AM supernatants after 3 h cultures (added at a final concentration of 0·5.

‡ BAL cytological findings of the 10 controls were given in Table 2.

Table 4. Production and activity of AM supernatants from CEP patients

Production of AM derived factors		CL modulation (ct/min) on eosinophils after various incubation times*	
Time course (culture time)	Dose (final concentration)	18 min	60 min
2 h	1/2	11206±1002	2154±1514
2 h	1/4	7616±823	1509±506
2 h	1/10	2758±1014	1090±915
3 h	1/2	10383±458	3189±1212
3 h	1/4	7569±814	3721±923
3 h	1/10	5120±900	1104±621

* Representative experiment on active AM factors tested on distinct normodense blood eosinophils derived from three different donors (each experiment was done in triplicate; mean±s.e.).

FMF analysis of surface IgE on blood and alveolar eosinophils

Comparative FMF studies were performed on eosinophil-rich suspensions from blood or lung in five cases of PIE Syndrome (Table 5).

Surface IgE and Fcε receptors were identified both on blood and tissue eosinophils. A large increase in the percentage of fluorescent cells was found after preincubation of blood cells with IgE (presence of free Fcε receptors on blood eosinophils). In contrast, only a slight increase was detected after preincubation of alveolar cells with IgE, due to the presence of occupied Fcε receptors on alveolar eosinophils (Table 5). Whereas the percentage of fluorescent cells after incubation with IgE and anti-IgE was close to 30% for all eosinophil populations tested; lung cells and in particular hypodense alveolar eosinophils from CEP patients showed an increased proportion of cells bearing cytophilic IgE.

Table 5. Flow-microfluorometry (FMF) analysis of surface IgE on blood and alveolar eosinophils, in five cases of PIE syndrome

	Percentage of surface IgE-bearing eosinophils*		
		Alveolar eosinophils	
Cell preincubation with:	Blood eosinophils Normodense	Normodense	Hypodense†
Medium+anti-IgE	13·2±7·5	23·2±7·5	31·0±4·1
IgE+anti-IgE	28·6±21·7	31·6±4·7	31·2±2·7

* The results are expressed as percentage of cells with fluorescence intensity above the threshold as previously detailed (Capron *et al.*, 1985). Mean±s.e.

† Hypodense alveolar eosinophils collected from the three CEP patients.

DISCUSSION

In chronic eosinophilic penumonia (CEP), histological findings include interstitial and intraalveolar eosinophils. The pathogenetic importance of these leucocytes is suggested by the presence in the interstitial tissue of mature (Carrington *et al.*, 1969; Morrissey *et al.*, 1975), degranulated eosinophils or eosinophilic granules phagocytosed by macrophages (Fox & Seed, 1980) and eosinophil rupture with interstitial edema (McEvoy, Donald & Edwards, 1978). The original aspect of the present work is the selective study of alveolar eosinophil subpopulations after separation on metrizamide gradients.

The distribution density of alveolar oesinophils varies according to the origin of the eosinophilic infiltrative pulmonary disease. A large proportion of BAL 'hypodense' alveolar eosinophils was only found in the three patients with active CEP. The potential harmful role of these cells has been postulated: such 'hypodense' eosinophils appear to be more activated than 'normodense' cells according to immunological, biochemical or metabolic criteria (Winquist *et al.*, 1982; Prin *et al.*, 1984) and can exhibit cytotoxic ability (Prin *et al.*, 1983; Capron *et al.*, 1984). The most striking change in CEP is a consolidation of the lung with widespread thickening of the interstitial tissue, bronchiolar lesions with occlusion of the lumen, a granulation tissue containing eosinophils, macrophages, multinucleated giant cells and local fibrin deposition. Granuloma and vasculitis with intravascular leucocyte rupture, platelet aggregation and coagulation in pulmonary vessels have also been described (Carrington *et al.*, 1969; Morrissey *et al.*, 1975; Fox & Seed, 1980; McEvoy *et al.*, 1978). Moreover, despite the usual dramatic resolution by steroid treatment (Carrington *et al.*, 1969), the process is not completely reversible. Previous reports have drawn attention to a residual peribronchial fibrosis (Carrington *et al.*, 1969; Perrault, Janis & Wolinsky, 1971). Other authors described irreversible airway alterations with occasional obliterans bronchiolitis (Carrington *et al.*, 1969; Morrissey *et al.*, 1975; Fox & Seed, 1980; McEvoy *et al.*, 1978). Thus, the therapeutic efficacy of corticosteroids may be limited. Although the exact mechanism remains undefined, steroids seem to depress blood hypodense eosinophil levels less than normodense levels (Prin *et al.*, 1983).

Heterogeneity of eosinophil density seems to be associated with functional heterogeneity (Prin *et al.*, 1983, 1984; Capron *et al.*, 1984; Winquist *et al.*, 1982). Thus, a predominant 'hypodense' alveolar eosinophil population in CEP may reflect an *in vivo* activation by mediators responsible for eosinophilic pulmonary infiltrates. Induction and regulation of this activation needs to be analysed. Cell cooperation signals and membrane immunological markers will be successively discussed.

Recent work has underlined the crucial role of resident AM populations in the release of leucocyte chemotactic factors in idiopathic pulmonary fibrosis (Hunninghake, Gadek & Lawley, 1981) and asthma (Gosset *et al.*, 1984). In the present work, eosinophils were able to produce increased levels of oxygen metabolites after stimulation by AM supernatants in the three tested cases of CEP, while AM supernatants of various controls, with different BAL-cell distribution, remain ineffective. Such membrane activation argues for an *in situ* cell cooperation and emphasizes the possible role of AM. Moreover, impaired *in vitro* oxidative metabolic activity of alveolar hypodense eosinophils possibly linked to previous *in vivo* activation has also been observed (Prin *et al.*, 1984). The mechanisms by which AM are able to modulate eosinophil function and the exact nature of locally generated factors are at present under investigation.

The role of IgE molecules in PIE syndrome is also evaluated. Eosinophil FcεR (Capron *et al.*, 1981; 1984) and surface IgE were demonstrated by using various techniques (Hubscher, 1975; Fujita *et al.*, 1975; Capron *et al.*, 1985). We have confirmed by flow microfluorometry that about 35% of alveolar eosinophils bear surface IgE. In contrast, free FcεR do exist on blood eosinophils. Studies on IgG molecules show similar large proportions if free FcγR on blood and tissue eosinophils (Capron *et al.*, 1985). These findings underline the possible functional role of cytophilic IgE molecules in inflammatory reactions in which eosinophils are prominent.

The existence of occupied FcεR on tissue eosinophils requires some comments. First, quantitatively, all tested patients with PIE syndrome presented elevated total serum IgE levels. Previous reports showed that the total IgE level in BAL fluids correlated with the total serum IgE level (Merrill, Naegel & Reynolds, 1980). However, previous immunofluorescent studies of lung

biopsy did not provide evidence of IgE deposition in interstitial sites (Fox & Seed, 1980; McEvoy *et al.*, 1978). The evaluation of local IgE in deep lung by sensitive assay in BAL fluid would be informative. Secondly, a higher IgE affinity for FcεR in tissue might be linked to the presence of complexed IgE antibodies. Previous reports have shown the existence of such complexes in or on nasal inflammatory eosinophils (Fujita *et al.*, 1975) which were able to promote cell activation such as peroxidase release (Takenata *et al.*, 1977). We have recently demonstrated an enhanced CL on tissue eosinophils after anti-human IgE stimuli (unpublished data) but their interaction with specific antigens remains to be defined. Indeed, patients with PIE syndrome presented, despite increased serum total IgE level, no atopic personal or family history, negative cutaneous prick-tests and negative specific IgE RAST (Pharmacia, Bois d'Arcy, France) against common allergens. Hypodense alveolar eosinophils from CEP patients showed an increased proportion of cells bearing such cytophilic IgE. McEvoy *et al.* (1978) previously evoked CEP as an IgE mediated hypersensitivity process and postulated that antigen-specific IgE bound on the eosinophil surface might promote tissue injury following eosinophil activation. This hypothesis may be extended to other situations in which an interaction exists between IgE molecules and tissue inflammatory eosinophils.

In conclusion, in contrast to blood eosinophils, a high percentage of hypodense eosinophils have been demonstrated in BAL of active CEP, while the alveolar eosinophil distribution appeared normal in other etiologies. In addition, supernatants of alveolar macrophages obtained from active CEP were able to stimulate eosinophil chemiluminescence arguing for a probable cell cooperation in the alveolar spaces. Moreover, the presence of cytophilic IgE on tissue eosinophils suggests a possible inductive role of IgE molecules in regulation or effector mechanisms in PIE syndrome.

The authors wish to thank Professor P. Godeau (CHU, Pitié-Salpêtrière, Paris) and Professor J. J. Lafitte, Dr E. Fournier (CHU, Hôpital Calmette, Lille) for allowing us to study their patients with PIE syndrome; G. Ovlaque for the preparation of purified human IgG; G. Marchandise, J. P. Papin and E. H. Sarkis for their excellent technical assistance, and C. Colson for her secretarial collaboration.

REFERENCES

BUTTERWORTH, A.E., STURROCK, R.F., HOUBA, V., MAHMOUD, A.A.F., SHER, A. & REES, P.H. (1975) Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature*, **256,** 727.

CAPRON, M., CAPRON, A., DESSAINT, J.P., TORPIER, G., JOHANSSON, S.G.O. & PRIN, L. (1981) Fc receptors for IgE on human and rat eosinophils. *J. Immunol.* **126,** 2087.

CAPRON, M., KUSNIERZ, J.P., PRIN, L., SPIEGELBERG, H.L., GOSSET, P., TONNEL, A.B. & CAPRON, A. (1985) Cytophilic IgE on human blood and tissue eosinophils: Detection by flow microfluorometry and relation to serum IgE. *J. Immunol.* **134,** 3013.

CAPRON, M., SPIEGELBERG, H.L., PRIN, L., BENNICH, H., BUTTERWORTH, A.E., PIERCE, R., OUAISSI, M.A. & CAPRON, A. (1984) Role of IgE receptors in effector function of human eosinophils. *J. Immunol.* **132,** 462.

CARRINGTON, C.B., ADDINGTON, W.W., GOFF, A.M., MADOFF, I.M., MARKS, A., SCHWABER, J.R. & GAENSEN, E.A. (1969) Chronic eosinophilic pneumonia. *New Engl. J. Med.* **280,** 787.

DESCAMPS-LATSCHA, B., NGUYEN, A.T., GOLUB, R.M. & FEUILLET-FIEUX, M.N. (1982) Chemiluminescence in microamounts of whole blood for investigation of the human phagocyte oxidative metabolism function. *Ann. Immunol. (Paris)*, **133 (3),** 349.

DURACK, D.T., ACKERMAN, S.J., LOEGERING, D.A. & GLEICH, G.J. (1981) Purification of human eosinophil-derived neurotoxin. *Proc. natn. Acad. Sci.* **78 (8),** 5165.

DURACK, D.T., SUMI, S.M. & KLEBANOFF, S.J. (1979) Neurotoxocity of human eosinophils. *Proc. natn. Acad. Sci.* **76,** 1443.

FOX, B. & SEED, W.A. (1980) Chronic eosinophilic pneumonia. *Thorax*, **35,** 570.

FREDENS, K., DAHL, R. & VENGE, P. (1982) The Gordon phenomenon induced by the eosinophil cationic protein and eosinophil protein X. *J. Allergy clin. Immunol.* **70,** 361.

FRIGAS, E., LOEGERING, D.A. & GLEICH, G.J. (1980) Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab. Invest.* **42,** 35.

FUJITA, Y., RUBINSTEIN, E., GRECO, D.B., REISMAN, R.E. & ARBESMAN, C.E. (1975) Antigen-antibody complexes in or on eosinophils in nasal secretions. *Int. Archs Allergy appl. Immun.* **48,** 577.

GLEICH, G.J., FRIGAS, E., LOEGERING, D.A., WASSOM, D.L. & STEINMULLER, D. (1979) Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* **123,** 2925.

GOSSET, P., TONNEL, A.B., JOSEPH, M., PRIN, L., MALLART, A., CHARON, J. & CAPRON, A. (1984) Secretion of chemotactic factor for neutrophils and eosinophils by alveolar macrophages from asthmatic patients. *J. Allergy clin. Immunol.* **74,** 827.

HOFFMAN, R.A. & HAUSEN, W.P. (1981) Immunofluorescence analysis of blood cells by flow cytometry. *Int. J. Pharmacol.* **3,** 249.

HUBSCHER, T. (1975) Role of the eosinophil in the allergic reactions. I. EDI, an eosinophil-derived

inhibitor of histamine release. *J. Immunol.* **114,** 1379.

HUNNINGHAKE, G.W., GADEK, J.E. & LAWLEY, T.J. (1981) Mechanism of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. *J. clin. Invest.* **68,** 259.

JONG, E.C. & KLEBANOFF, S.J. (1980) Eosinophil-mediated mammalian tumor cell cytotoxicity: role of the peroxidase system. *J. Immunol.* **124,** 1949.

JONG, E.C., MAHMOUD, A.A.F. & KLEBANOFF, S.J. (1981) Peroxidase-mediated cytotoxicity to schistosomula of *Schistosoma mansoni*. *J. Immunol.* **126,** 468.

MCEVOY, J.D.S., DONALD, K.J. & EDWARDS, R.L. (1978) Immunoglobulin levels and electron microscopy in eosinophilic pneumonia. *Am. J. Med.* **64,** 529.

MERRILL, W.W., NAEGEL, G.P. & REYNOLDS, H.Y. (1980) Reaginic antibody in the lung lining fluid. Analysis of normal human bronchoalveolar lavage fluid IgE and comparison to immunoglobulins G and A. *J. Lab. clin. Med.* **96,** 494.

MORRISSEY, W.L., GAENSLER, E.A., CARRINGTON, C.B. & TURNER, H.G. (1975) Chronic eosinophilic pneumonia. *Respiration*, **32,** 453.

PERRAULT, J.L., JANIS, M. & WOLINSKY, H. (1971) Resolution of chronic eosinophilic pneumonia with corticoid therapy. *Ann. Intern. Med.* **74,** 951.

PRIN, L., CAPRON, M., TONNEL, A.B., BLETRY, O. & CAPRON, A. (1983) Heterogeneity of human peripheral blood eosinophils: variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. *Int. Archs Allergy appl. Immun.* **72,** 336.

PRIN, L., CHARON, J., CAPRON, M., GOSSET, P., TAELMAN, H., TONNEL, A.B. & CAPRON, A. (1984) Heterogeneity of human eosinophils. II. Variability of respiratory burst activity related to cell density. *Clin. exp. Immunol.* **57,** 735.

SPRY, C.J.F., TAI, P.C. & DAVIES, J. (1983) The cardiotoxicity of eosinophils. *Postgraduate Med. J.* **59,** 147.

TAI, P.C., HAYES, D.J., CLARK, J.B. & SPRY, C.J.F. (1982) Toxic effects of human eosinophil secretion products on isolated rat heart cells *in vitro*. *Biochem. J.* **204,** 75.

TAKENATA, T., OKUDA, M., KAWABORI, S. & KUBO, K. (1977) Extracellular release of peroxidase from eosinophils by interaction with immune complexes. *Clin. exp. Immunol.* **28,** 56.

WINQUIST, I., OLOFSSON, T., OLSSON, I., MAYPERSSON, A. & HALLBERG, T. (1982) Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology*, **47,** 531.