Involvement of Immunoglobulin E in the Secretory Processes of Alveolar Macrophages from Asthmatic Patients

MICHEL JOSEPH, ANDRÉ-BERNARD TONNEL, GÉRARD TORPIER, and ANDRÉ CAPRON, *Centre d'Immunologie et de Biologie Parasitaire (Institut National de la Santé et de la Recherche Médicale U 167, Centre National de la Recherche Scientifique ERA 422), Institut Pasteur, Lille, France*

BERNARD ARNOUX and JACQUES BENVENISTE, *Institut National de la Santé et de la Recherche Médicale U200, Université de Paris-Sud, Clamart, France*

ABSTRACT Alveolar macrophages from nonatopic donors were passively sensitized with allergen-specific IgE antibody from the serum of asthmatic patients. A selective release of 4–8% of the lysosomal β-glucuronidase of these cells occured within 30 min of contact with the related allergen or with anti-human IgE antibody, in the absence of any mast or basophil cells. The cell reactivity was dependent on the interaction of macrophages with IgE, as shown by the disappearance of the allergen-induced enzyme release after heating or IgE-immune adsorption of the sensitizing serum, but not after IgG-adsorption. Alveolar macrophages from asthmatic patients behaved similarly to passively sensitized normal macrophages. Contact with the related allergen or with anti-IgE antibody induced the same percentage of enzyme release, demonstrating that these cells possess allergen-specific IgE bound on their surface. 18% of them formed rosettes with anti-IgE-coated sheep erythrocytes, and 15–22% with allergen-coated erythrocytes, but lost this property after preincubation with the specific allergen. The presence of IgE-specific receptors on the macrophage surface was demonstrated both at the ultrastructural level with immunoperoxidase labeling, and at low magnification by the formation of 15–18% rosettes with human IgE-coated erythrocytes. The formation of such rosettes was inhibited after incubation of alveolar phagocytes with aggregated myeloma IgE. On the basis of these observations, the participation of the alveolar macrophages in IgE-mediated pulmonary hypersensitivity must be considered. Its precise involvement requires, however, further investigations.

INTRODUCTION

The first evidence of a direct interaction of IgE antibody with the macrophage was obtained when studying an antibody-dependent cell-mediated cytotoxicity against larvae of *Schistosoma mansoni* (1–3) and *Dipetalonema viteae* (4). These experiments showed that such an interaction induced the activation of normal mononuclear phagocytes from man and animals into cytotoxic effector cells. The demonstration of an Fc receptor for IgE on the surface of macrophages from different species was then reported (5–10), and IgE dimers were shown to represent the minimal molecular size of IgE aggregates necessary to stimulate various metabolic parameters of the cells (7). These observations have opened new concepts in the activation mechanisms of macrophages, especially in human pathologic situations where an interaction between IgE and macrophages is likely to occur, particularly in atopic diseases.

In a previous paper, we described the stimulation of normal alveolar macrophages either by myeloma IgE or by allergen-specific IgE from serum of asthmatic patients that triggered lysosomal enzyme release, neutral protease synthesis, or superoxide anion generation (11). Here, using the specific release of lysosomal enzymes with anti-IgE or allergens as a screening parameter of cell reactivity, electron microscopy

Address all correspondence to Dr. M. Joseph, Institut Pasteur, B.P. 245, 59019 Lille Cedex, France.

Received for publication 7 April 1982 and in revised form 8 October 1982.

and rosette formation with anti-IgE- or allergen-sensitized erythrocytes, we show that alveolar macrophages from asthmatic patients have surface-bound IgE molecules in vivo, and are specifically triggered by contact with the sensitizing allergens.

METHODS

Patients. The control population for the collection and the in vitro culture of normal alveolar macrophages consisted of 45 nonatopic patients (18 women and 27 men, mean age 35 yr), devoid of any bronchospastic manifestation, showing negative cutaneous prick tests and, for 20 of them, a mean value of 240±182 IU total IgE/ml of serum (normal range: 50–500 IU/ml).

21 asthmatic patients (7 women and 14 men, mean age 34 yr) were selected for macrophage isolation and triggering. Allergic asthma was confirmed by clinical history, by respiratory function tests, and with at least one positive cutaneous test to the following allergens: house dust mite *Dermatophagoides pteronyssinus* (DP),[1] mixed grass pollens (GP) (Laboratoires Stallergènes, 94260 Fresnes, France) and *Aspergillus fumigatus* (Asp) (Centre d'Immunologie, Institut Pasteur, Lille, France). The mean level of serum IgE, measured in all cases, was 1,528±1,469 IU/ml. The positivity of the cutaneous tests was correlated with specific IgE assayed by a radioallergosorbent test (RAST, Pharmacia Fine Chemicals, Uppsala, Sweden). In the asthmatic patients, the bronchoalveolar lavage was carried out at least 2 wk after the last acute bronchospastic reaction, and only if corticosteroids or β-adrenergic drugs had been discontinued for at least 1 wk. No treatment was given to prevent bronchospasm. 7 out of 21 patients showed a discrete bronchospasm within a few minutes after the fibroscopic procedure, but one or two puffs of Salbutamol (Glaxo, London, United Kingdom) were sufficient to stop the side effect. Both atopic and nonatopic patients gave appropriate informed consent for lavage and for experimental use of the alveolar cells.

Macrophage isolation. Alveolar lavages were performed under fiberoptic bronchoscopy (BF2, Olympus Corporation of America, New Hyde Park, NY) by injection of 50-ml aliquots of 250 ml physiologic saline in a subsegmental bronchus. The first 50 ml, corresponding to bronchial lavage, were recovered and discarded. The following four injections, considered as the actual lavage, were recovered and centrifuged at 200 *g* for 10 min at 4°C. Cell counts and identifications were carried out at all steps, using Giemsa and toluidine blue staining to evaluate accurately mast cell contamination. The pellet was suspended to a cell concentration of 10^6/ml in Eagle's minimum essential medium (MEM) (Institut Pasteur Production, Paris, France) supplemented with 10% heat-inactivated foetal calf serum (Flow Laboratories, Inc., Irvine, Scotland), 100 IU penicillin/ml and 50 μg streptomycin/ml (Specia, Paris, France). Cells (2.5×10^6) were 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 nonadherent cells, alveolar macrophages, purified up to 97%, were either immediately tested or further incubated overnight in the same medium. The number of adherent cells in the petri dishes was calculated by the difference between the number of cells at the beginning of the incubation and the number of discarded cells during the washing procedures (generally 50%).

Macrophage sensitization and stimulation. After overnight culture, the alveolar macrophages from normal donors were sensitized directly in the petri dishes by a 30-min incubation at 37°C either in 10 μg/ml human myeloma IgE protein (a generous gift from Dr. H. Bazin, Brussels), or in serum from asthmatic patients (20% in MEM). After thorough washing, the cells were incubated for 30 min with 10 μl goat anti-human IgE/ml phenol red-free Hanks' balanced salt solution (colorless HBSS), or with the allergen corresponding to the serum sensitivity (generally 100 ng dry wt/ml colorless HBSS). Supernatants were assayed for β-glucuronidase activity with *p*-nitrophenyl-β-glucuronide as substrate (12). The intracellular content of β-glucuronidase was measured by the same procedure after cell lysis with Triton X-100 (0.05% in Tris $MgCl_2$ 0.01 M pH 7.4). For both supernatant and cell lysate, the volume of the assay corresponded to $2.0–2.5 \times 10^5$ macrophages. Histamine release and total cell content was quantified by a fluorometric procedure (13). Positive controls consisted of human basophils, isolated with the leukocytes of dextran-sedimented blood, or rat peritoneal mast cells, degranulated by incubation with 1 μmol ionophore A23187 (Eli Lilly & Co., Indianapolis, IN).

The freshly isolated (2 h) alveolar macrophages from asthmatic subjects were immediately triggered, directly in the petri dishes, by 10 μl anti-IgE serum/ml, or by the allergen(s) responsible for the observed pathology. As above, for the normal macrophages, the β-glucuronidase release was used as a parameter of cell triggering. Macrophages from normal donors, handled in the same conditions, served as controls.

The absence of cell lysis during cell triggering was appreciated by the level of the cytoplasmic marker lactate dehydrogenase (LDH) in the supernatant, assessed with LDH Biochemica Test (Boehringer, Mannheim, West Germany).

Sera from asthmatic patients and Ig depletion. For the passive sensitization of normal macrophages, sera from asthmatic patients were selected according to their IgE level, measured by a radioimmunosorbent test for total IgE and radioallergosorbent test for specific IgE (Pharmacia Fine Chemicals). IgG or IgE depletion was carried out by solid-phase immunoadsorption with anti-Ig Sepharose 4B (Pharmacia Fine Chemicals).

Rosette assays. Sheep erythrocytes (SRBC) were treated according to the method of Spiegelberg and Melewicz (14), by successive incubation with trypsin, pyruvic aldehyde, and formaldehyde. The fixed erythrocytes were coated either with purified human myeloma IgE protein (IgE-SRBC) or with rabbit IgG anti-human IgE (Dako, Copenhagen, Denmark) (anti-IgE-SRBC). For rosette assays, 1×10^5 alveolar macrophages, recovered at the end of the 2-h adherence phase by a rubber policeman at 0°C, were mixed with 1×10^6 indicator erythrocytes in a final volume of 200 μl, centrifuged for 5 min at 100 *g* and incubated for 1 h, these different steps being carried out at 4°C. The cells were then gently mixed and counted in Fuchs-Rosenthal hemocytometer. The percentage of rosette-forming macrophages was appreciated on 200 cells. Macrophages with three or more adherent erythrocytes were considered as rosettes.

An allergen-specific rosette assay was performed in the same way after incubation of the fixed erythrocytes with one of the following allergens: *Dermatophagoïdes pteronyssinus* (DP-SRBC), mixed grass pollens (GP-SRBC), or *Aspergillus fumigatus* (Asp-SRBC). For allergen- as well as IgE-rosettes, spontaneous rosette formation was measured with control

[1] *Abbreviations used in this paper*: Asp, *Aspergillus fumigatus*; DP, *Dermatophagoïdes pteronyssinus*; GP, grass pollens; LDH, lactate dehydrogenase; PAF-acether, platelet activating factor; SRBC, sheep erythrocytes.

erythrocytes sensitized, after fixation, with L-lysine 0.1 M, and subtracted from specific rosettes. In some experiments, alveolar macrophages were first incubated with human IgE (10 μg/ml) aggregated with dimethylsuberimidate (15, 16) or with the related allergen (5 μg/ml) for 15 min at 0°C, before the addition of indicator erythrocytes.

Electron microscopy. Immediately after bronchoalveolar lavage, macrophages were washed in HBSS, and fixed for 30 min with glutaraldehyde 0.4% in cacodylate buffer at 4°C. After washing with the same buffer, they were incubated overnight with L-lysine 0.1 M in phosphate-buffered saline 0.1 M pH 7.4 at 37°C. McCoy cell line (a gift from Dr. S. Darougar, Institute of Ophthalmology, University of London) was used as negative control for IgE detection, since these cells show no Fc receptors and are easily identified by endogenous production of virus particles. Rat mast cells were used as a positive control after isolation from the nonadherent population of peritoneal cells (17). Both McCoy and mast cells were fixed and treated with L-lysine as for alveolar macrophages. After thorough washing, rat mast cells were incubated with rat myeloma IgE (IR 162 myeloma protein) for 1 h at 37°C, washed, and subsequently treated, in the same conditions, with rabbit IgG anti-rat IgE (Dr. H. Bazin, Brussels). Human macrophages and McCoy cells were directly incubated with rabbit IgG anti-human IgE (Dako). All three categories of cells were then mixed in the same tube for the identification of IgE-bearing cells, with commercial peroxidase-labeled sheep Fab anti-rabbit IgG (Institut Pasteur Production). The peroxidase activity was visualized by incubation with diaminobenzidine (Fluka, Buchs, Switzerland) and H_2O_2 for 5 min (18). After three washings with Tris-HCl buffer 0.01 M, the cells were postfixed in 1% osmium tetroxide for 60 min, dehydrated in acetone, and embedded in araldite. Sections, contrasted with lead hydroxide, were examined with a Hitachi HU 12 electron microscope (Hitachi Ltd., Tokyo, Japan).

RESULTS

Cell populations. Due to a slightly better fluid recovery, the average number of cells in nonatopic individuals was 32.9±23.7 × 10^6, while it was 19.4±17.3 × 10^6 in atopic patients. Cell percentages, before and after plating, have been evaluated and are presented in Table I. Special attention was payed to the presence of mast cells: on a few occasions the crude cell population, fixed and stained as soon as it was recovered from the lung, contained 0.045% intact mast cells, but most of the alveolar lavages, even in asthmatic patients, were devoid of mast or basophiloid cells on morphological or staining basis. At the end of the 2-h adherence phase, no more mast cells could be seen in any cultures. Remaining cells, 98% viable by trypan blue dye exclusion, were esterase-positive intact alveolar macrophages that in no case could be mistaken for mast cells.

Sensitization of macrophages from normal donors by IgE. A 30-min incubation of alveolar macrophages from normal donors, either with purified human myeloma IgE protein, or with the serum of asthmatic patients, induced cell sensitization. Indeed, lysosomal enzyme release occurred following a subsequent 30-min incubation of the sensitized cells with anti-IgE or with the allergen related to the sensitivity of the donor (Fig. 1). Unrelated allergens had no effect on IgE- or allergic serum-sensitized macrophages. Extracellular enzyme was produced by a secretory process, and was not the consequence of cell death or lysis, as judged by the absence of LDH in supernatants. The involvement of IgE antibody in the cell triggering by asthmatic sera was evidenced by the disappearance of significant enzyme exocytosis with allergens after specific IgE depletion, either by heating or by IgE adsorption of the sera. IgG adsorption had no inhibitory effect on the cell stimulation induced by specific allergens (Table II).

Allergenic stimulation of macrophages from asthmatic donors. Incubation of alveolar macrophages from asthmatic patients with anti-IgE or with allergens related to the patient sensitivity induced enzyme release without in vitro presensitization of the cells (Table III). Macrophages from normal individuals also showed a limited, but significant enzyme release with anti-IgE treatment. Allergens, however, had no triggering action on these normal cells. The allergen concentration uniformly used with all three preparations was 100 ng dry wt/ml final culture medium. With *D. pteronyssinus*, maximal enzyme release was obtained between 100 and 500 ng dry wt/ml, the shape of the dose-response curve being very similar to that ob-

TABLE I
*Alveolar Cell Population before and after the Adherence Stage**

	Cell population	Macrophages	Lymphocytes	Eosinophils	Neutrophils	Mast cells
Normal donors	Before plating	92.3±4.5	6.3±3.2	0.2±0.2	1.2±1.2	0
n = 15	After plating	99.3±2.1	0.4±0.5	0.1±0.1	0.2±0.2	0
Asthmatic patients	Before plating	90.7±4.9	5.6±3.7	2.7±3.7	0.9±1.1	0.045±0.03
n = 18	After plating	96.8±3.1	2.6±3.1	0.2±0.2	0.4±0.3	0

* Expressed as mean percentages±1 SD. Giemsa staining was applied to the total cell populations after cytocentrifugation, and to the adherent cells directly in the petri dishes.

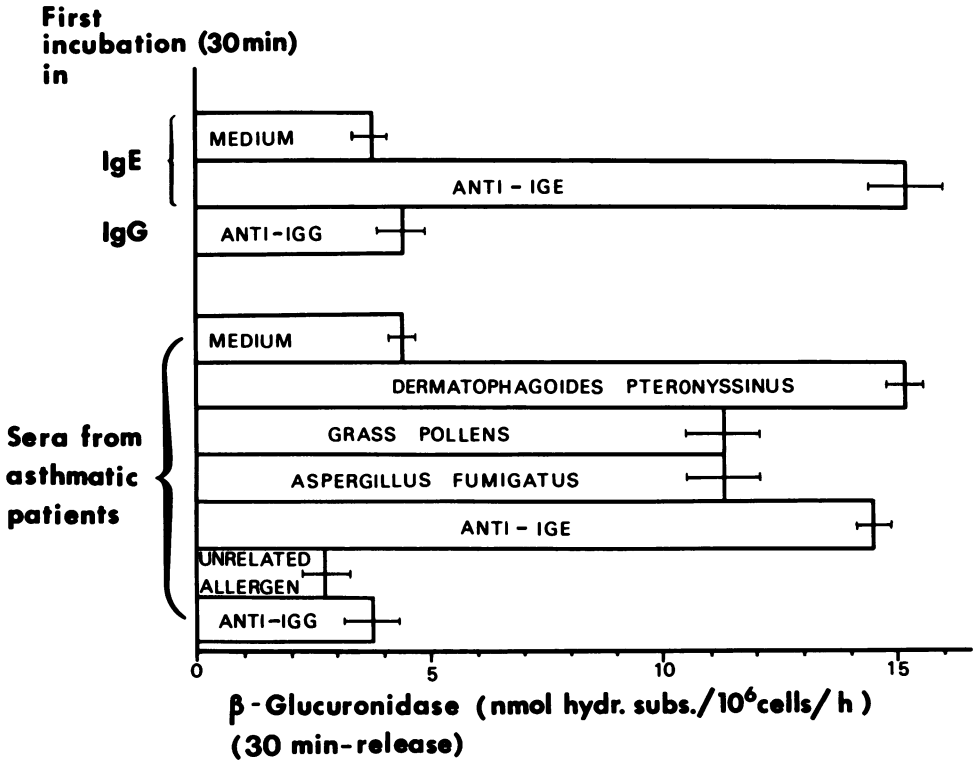


FIGURE 1 Lysosomal enzyme release from 24-h normal alveolar macrophages passively sensitized in vitro by dimethylsuberimidate-aggregated IgE, heat-aggregated IgG, or sera from asthmatic patients, and triggered by a further 30-min incubation with the agents indicated in each column (mean±1 SD of 12 experiments). Hydr. subs., hydrolysed substrate.

served for histamine release by mast cells or basophils (Fig. 2). However, no histamine could be demonstrated in the macrophage supernatants or in total cell lysates, while human blood basophils or rat mast cells presented a high histamine release with the ionophore A23187.

TABLE II

*Evidence for the Role of IgE in the β-Glucuronidase Release from Normal Human Alveolar Macrophages Incubated with Serum from Asthmatic Patients**

First incubation	Second incubation			Intracellular enzyme
	Anti-IgE	Specific allergen	Control medium	
Serum				
untreated‡	16.6±4.7	10.2±2.8	3.2±0.6	318.0±1.4
56°C, 3 h	2.6±0.8§	2.8±0.8§	3.2±1.0	296.5±12
anti-IgE adsorbed‖	3.8±2.5§	5.3±2.4§	3.4±0.3	305.0±2.8
anti-IgG adsorbed¶	17.6±4.2	14.1±2.7	2.3±0.6	314.5±3.5

* Normal human macrophages, after overnight culture, were first incubated for 30 min in native or treated serum from asthmatic patients. After washing, the cells were incubated for a further 30-min period in heat-inactivated goat serum anti-human IgE (10 μl/ml HBSS), or in 100 ng specific allergen, or in HBSS alone. β-Glucuronidase release is expressed as nanomoles of hydrolysed substrate per 10^6 cells per hour (three experiments in triplicate).

‡ Unadsorbed sera: 8.6 mg IgG/ml and 4.6 μg IgE (2,000 IU)/ml.

§ Comparison with untreated serum: $P < 0.001$.

‖ Solid-phase adsorbed sera: <0.025 μg IgE (<10 IU)/ml.

¶ Solid-phase adsorbed sera: 0.4 mg IgG/ml.

TABLE III

*Extra- and Intracellular Levels of β-Glucuronidase from Alveolar Macrophages**

	Normal donors			Asthmatic patients		
		β-glucuronidase level			β-glucuronidase level	
30-min incubation with	*n*	Extracellular	Intracellular	*n*	Extracellular	Intracellular
Control medium	6	2.0±0.3	305.4±31.1	8	3.4±0.8	368.4±21.8
Anti-IgG antiserum	2	4.5±0.5	322.6±17.3	2	3.2±0.2	368.3±19.0
Anti-IgE antiserum	6	8.4±0.5‡	333.1±22.5	8	18.2±2.9‡	388.3±18.2
D. pteronyssinus§	—	—	—	6	14.0±0.6‡	389.9±23.3
GP§	—	—	—	5	10.9±0.8‡	367.4±43.8
A. fumigatus§	—	—	—	3	13.0±1.4‡	373.8±23.6
Unrelated allergen§	9	1.4±0.2	342.8±27.6	6	2.5±0.3	360.9±25.7

* Nanomoles of hydrolysed substrate per 10^6 cells per hour (mean±SD of *n* experiments in quadruplicates on 2-h alveolar macrophages).

§ Allergens, 100 ng dry wt/ml.

‡ Comparison with experiments using anti-IgG, control medium, or unrelated allergen, $P < 0.001$.

Electron microscopic observation of membrane IgE. IgE-specific peroxidase labeling of alveolar macrophages was obtained with both atopic and nonatopic cells as soon as isolated from the bronchoalveolar lavage in two reproducible experiments (Fig. 3). With nonatopic macrophages, the IgE label was more diffuse and uniformly spread on the cell surface, whereas on macrophages from asthmatic patients the peroxidase activity was mainly localized on the cell processes. Controls showed an absence of staining for McCoy

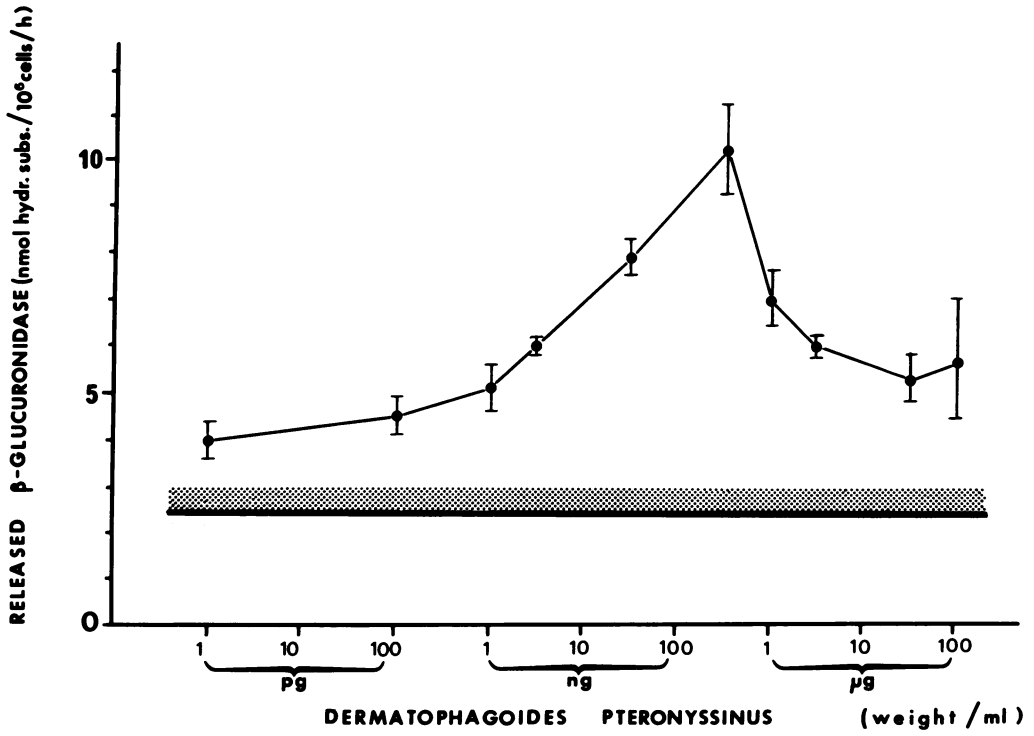
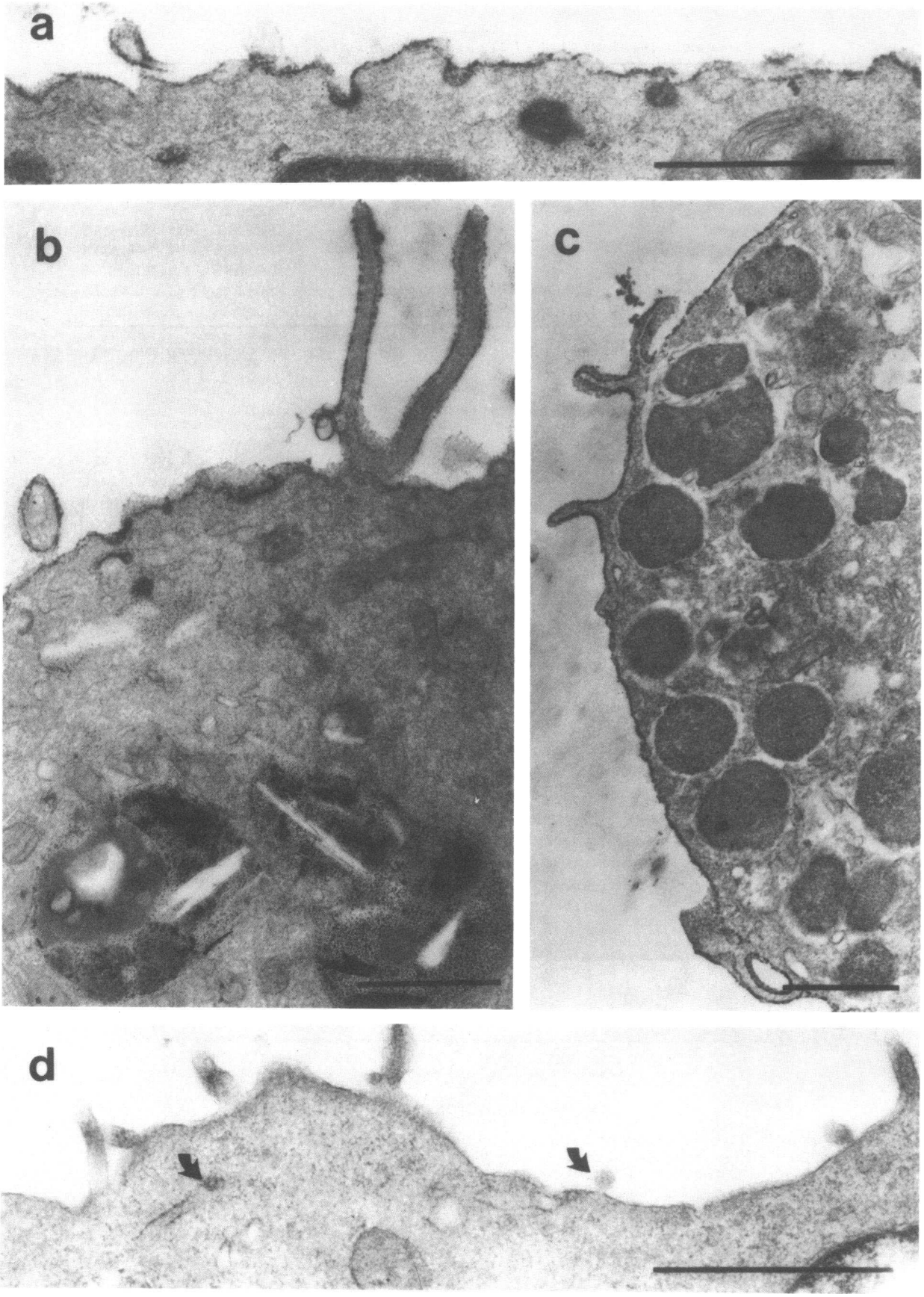


FIGURE 2 β-Glucuronidase release from macrophages, 2 h after bronchoalveolar lavage from patients allergic to house dust. The macrophages were incubated in vitro for 30 min with various concentrations of *D. pteronyssinus* allergen (mean±1 SD of four experiments in duplicate). The grey horizontal line indicates spontaneous enzyme release by cells in medium alone + 1 SD. Hydr. subs., hydrolysed substrate.


a
b
c
d

cells, and highly positive labeling for IgE-incubated rat mast cells. These observations confirmed, at the ultrastructural level, the IgE-labeling of macrophage populations analyzed under light microscopy after Giemsa or toluidine blue staining.

Anti-IgE and allergen-specific rosettes. The percentages of human alveolar macrophages forming rosettes with SRBC, anti-IgE-SRBC, and allergen-coated SRBC were compared in 10 controls and 11 asthmatic patients (Fig. 4). While the mean percentage of rosette-forming macrophages with SRBC was the same in both groups, the number of anti-IgE-rosettes was increased threefold in asthmatic patients when compared with control alveolar macrophages.

In eight patients suffering from mite allergic asthma, the percentage of DP-SRBC rosettes was 19.7±1.2%. For the patients with GP allergy or *A. fumigatus* sensitivity, it was 15.5 and 22.2% for GP-SRBC and Asp-SRBC rosettes, respectively.

Rosette inhibition after incubation with IgE or allergen. In two asthmatic patients, a prior incubation of the alveolar macrophages with aggregated IgE induced a 50% reduction of the IgE-rosettes, whereas pretreatment with soluble allergens (5 μg/ml), at 4°C to avoid cell triggering, was also followed by an inhibition of allergen-specific rosettes (Fig. 5). Similarly, pretreatment of macrophages from asthmatic patients with anti-IgE antibody at 4°C completely inhibited the allergen-specific rosettes (96.7%) whereas anti-IgG pretreatment had no significant effect (16.7% inhibition of allergen-rosettes). Finally, an incubation with aggregated IgE induced no change in the number of anti-IgE rosettes with macrophages from asthmatic patients but a significant increase (300%) with normal cells.

DISCUSSION

In this study, we provide evidence that IgE antibody is capable of stimulating alveolar macrophages when asthmatic patient sera are used for the passive sensitization of normal cells, leading to a metabolic burst not only with anti-IgE as the triggering signal, but also with specific allergens. These results extend to 45 patients the previously reported observation (11) of the binding of allergen-specific IgE to the macrophage surface, subsequently triggered to selectively secrete lysosomal enzymes by a contact with allergen or anti-IgE antibody. They also confirm the close relationship between the specificity of the triggering allergens and that of the IgE antibody, assayed by RAST, present in the sensitizing sera.

The crucial point for the interpretation of these results is, however, the possible presence and participation of mast or basophiloid cells in the process described here. Alveolar cells were collected after discarding the contaminant bronchial cells of the first 50 ml of the lavage. The morphological determination of cells, after Giemsa and toluidine blue staining, showed very low level of mast cell contamination in the crude populations, and, on the very rare occasions when some were visible, they were no longer present after the 2-h adherence stage. Furthermore, the absence of any histamine release during IgE-anti-IgE triggering of the cells, when significant enzyme release was measured, makes the participation of this cell type in the observed effect unlikely.

The molecular form of the IgE involved was not investigated here. However, monomeric as well as polymeric IgE were both demonstrated to bind to rat macrophages at 37°C, even if dimers and higher aggregates showed a better affinity than monomers (7). In the present data, the possible participation of IgE immune complexes in the sera from asthmatic patients cannot be ruled out nor their possible spontaneous aggregation by the freezing conditions of storage. These points will be considered in the near future.

The participation of allergen-specific IgG in the passive sensitization of normal macrophages is doubtful, since the cell reactivity to the allergens was unchanged after the immune adsorption of IgG, whereas heating the sensitizing sera suppressed it. Furthermore, the time course of the process (30 min) makes a major role for IgG unlikely, since, as was shown with rat peritoneal macrophages, the IgG-anti-IgG triggering requires at least 6 h contact to reach the level of cell sensitization obtained within 30 min in the IgE-anti-IgE procedure (19). In fact, with passively sensitized alveolar macrophages, a 30-min incubation with anti-IgG did not induce a significantly higher level of enzyme release than incubation with control medium (Fig. 1). The short duration of the incubation also accounts for the apparently low level of enzyme release, comprised between 4 and 10% of the total β-glucuronidase content. The anti-IgE or the allergen-induced release could be increased by a longer incubation, but it was accompanied by a concomitant increase of the intracellular synthesis of the enzyme.

FIGURE 3 Electron microscopic demonstration of IgE on the surface of (a) an alveolar macrophage from a normal donor; (b) an alveolar macrophage from an asthmatic patient; and (c) an IgE-incubated rat peritoneal mast cell. As control, (d), absence of peroxidase-labeling on a Fc receptor-free McCoy cell with characteristic intracellular and budding virus particles (arrows). Bars = 1 μm.

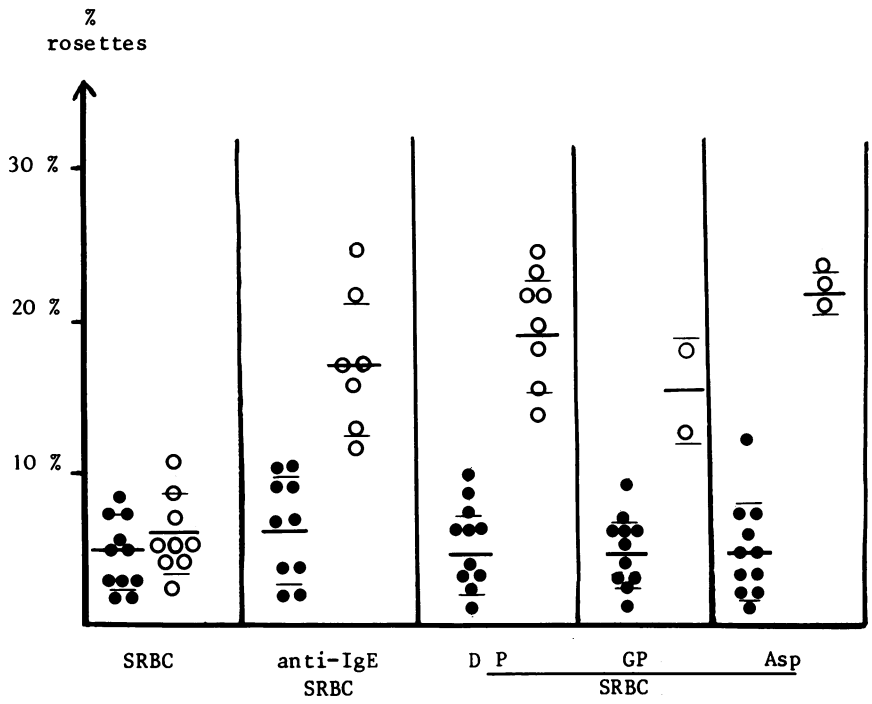


FIGURE 4 Rosette formation between 2-h alveolar macrophages and fixed sheep erythrocytes (SRBC) sensitized with anti-human IgE antibody, *D. pteronyssinus* (DP), GP, and *A. fumigatus* (Asp) allergens (macrophages from normal (●) or asthmatic (○) patients).

However, the stimulated release was 3–10 times greater than the spontaneous release by resting macrophages.

Electron microscopy and rosette assay with anti-IgE coated erythrocytes evidenced surface bound IgE in alveolar macrophages obtained from asthmatic patients with a typical history of allergic disease, positive skin tests, and high levels of allergen-specific IgE. The relatively low percentages of macrophages showing IgE receptors in this paper is in accordance with the

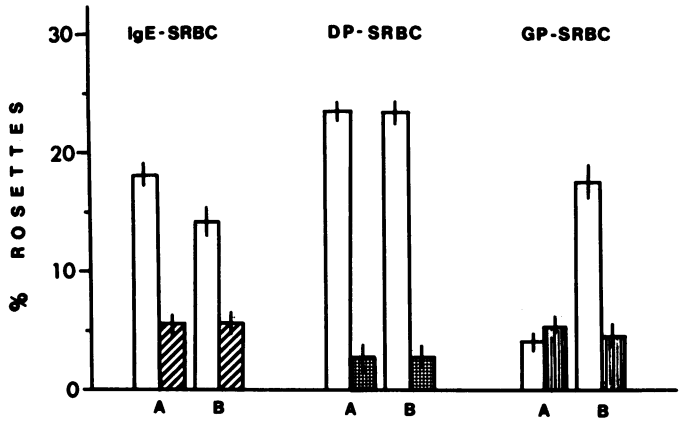


FIGURE 5 Inhibition of rosette formation between IgE- or allergen-sensitized erythrocytes and 2-h alveolar macrophages from two asthmatic patients by preincubation of the macrophages with aggregated IgE (diagonal bars columns), *D. pteronyssinus* (dotted columns) and GP (vertical bars columns) allergens, compared to rosette formation by macrophages preincubated with medium alone (empty columns). (A) Patient allergic to *D. pteronyssinus*, and (B) patient allergic to *D. pteronyssinus* and to GP (mean of one experiment in triplicate).

recent determination by Melewicz et al. (20) for alveolar macrophages from healthy donors, with 8% IgE rosettes. The reason for such a reduced percentage, compared with the 64% of macrophages with IgG receptors, merits further investigation. In this respect, the reported development of mast cells, among mononuclear cell populations, by long-term cultivation of rat peritoneal cells (21) must be kept in mind, even if this observation has to be substantiated. Nevertheless, as established for blood monocytes in patients with severe allergic disorders (22), our results show an increased number of IgE Fc receptors and a higher density of bound IgE on alveolar macrophages from asthmatic patients. These cells demonstrated a very fast reaction to anti-IgE triggering, whereas the incubation with anti-IgG was inefficient in the same time-lapse. Furthermore, the enzyme release from the cells was specifically obtained upon contact with the allergen related to the patient sensitivity: house dust mites, GP, or *A. fumigatus*. The narrow antigen specificity of the reactivity of macrophages from asthmatic patients focuses the attention on this cell type in lung allergy.

The direct demonstration of an IgE antibody involvement in the allergen-induced enzyme release by macrophages from asthmatic patients is not given in the experiments reported here, as it is for passively sensitized normal macrophages. However, the time course of the β-glucuronidase release in the presence of the specific allergens is very similar to that observed with anti-IgE, and its level significantly higher than after treatment by anti-IgG serum. The inhibition of allergen-specific rosettes, not only by preincubation with the related allergen but also by anti-IgE pretreatment, provides evidence that IgE antibody is the main isotype participating in the cell-triggering process with allergens. Experiments in progress on fractionated macrophage populations will give indications on the precise involvement of each immunoglobulin class in this mechanism.

In response to different stimulations, alveolar macrophages produce a large variety of mediators, such as hydrolytic enzymes (23–26), superoxide anion (11), platelet-activating factor (PAF-acether) (27), slow-reacting substances (SRS) (28) and other arachidonic acid metabolites (29–31). In this work, the choice of only lysosomal enzyme release as a parameter of cell stimulation was inspired by its practicality in a large screening study. Nevertheless, it allowed the demonstration of a significant difference in the behavior of alveolar macrophages from normal vs. asthmatic donors during the interaction of IgE antibody with the specific allergens. However, the release of this enzyme does not give information on the pathogenic role of the alveolar macrophage stimulation in the onset of bronchoconstriction. On the one hand, SRS from alveolar macrophages might intervene in this process. On the other hand, the bronchomotor action of PAF-acether is well documented (32). PAF-acether release by alveolar macrophages from asthmatic patients was observed in the stimulation process described here (Arnoux et al., submitted for publication). Investigations on the production of the other mediators cited above in the same pathological situation will provide new information on their role in regulating the functions of other cells (33, 34), in increasing the cytotoxic properties of the pulmonary phagocytes, as well as in participating in the inflammatory reactions and tissue damage of the lung pathology.

Together with the IgE-dependent mechanism of specific killing by mononuclear phagocytes of at least two different metazoan parasite larvae (1–4) the interaction reported here between IgE antibody and alveolar macrophages opens an original approach to the triggering processes of this type of phagocytes. It gives indications of a new role for this immunoglobulin class, up till now restricted to anaphylactic functions at the basophil or mast cell level. Finally, besides the role of mast cells in the bronchial submucosa, the participation of the alveolar macrophage in the respiratory allergy must therefore be taken into account for full description of the lung hypersensitivity.

ACKNOWLELDGMENTS

We are very grateful to Professor Hervé Bazin for the generous supply of purified human myeloma IgE, and to Dr. Eric Fournier for his fruitful participation in the bronchoalveolar lavages. We thank Ms. Sylvie Odent and Mr. Han Vorng for their excellent technical assistance, Mr. Ray Pierce for revising the manuscript and Mrs. Claudine Colson and Christiane Zeiske for their secretarial collaboration.

REFERENCES

1. Capron, A., J. P. Dessaint, M. Capron, and H. Bazin. 1975. Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature (Lond.).* **253:** 474–475.
2. Capron, A., J. P. Dessaint, M. Joseph, R. Rousseaux, M. Capron, and H. Bazin. 1977. Interaction between IgE complexes and macrophages in the rat: a new mechanism of macrophage activation. *Eur. J. Immunol.* **7:** 315–322.
3. Joseph, M., A. Capron, A. E. Butterworth, R. F. Sturrock, and V. Houba. 1978. Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula *in vitro*: induction by immune complexes containing IgE and *Schistosoma mansoni* antigens. *Clin. Exp. Immunol.* **33:** 48–56.
4. Haque, A., M. Joseph, M. A. Ouaissi, M. Capron, and A. Capron. 1980. IgE antibody-mediated cytotoxicity of rat macrophages against microfilaria of *Dipetalonema viteae in vitro*. *Clin. Exp. Immunol.* **40:** 487–495.
5. Dessaint, J. P., G. Torpier, M. Capron, H. Bazin, and A. Capron. 1979. Cytophilic binding of IgE to the macrophage. I. Binding characteristics of IgE on the surface of macrophages in the rat. *Cell. Immunol.* **46:** 12–23.

6. Dessaint, J. P., A. Capron, M. Joseph, and H. Bazin. 1979. Cytophilic binding of IgE to the macrophage. II. Immunologic release of lysosomal enzyme from macrophages by IgE and anti-IgE in the rat: a new mechanism of macrophage activation. *Cell. Immunol.* **46:** 24–34.
7. Dessaint, J. P., B. H. Waksman, H. Metzger, and A. Capron. 1980. Cytophilic binding of IgE to the macrophage. III. Involvement of cyclic GMP and calcium in macrophage activation by dimeric or aggregated rat myeloma IgE. *Cell. Immunol.* **51:** 280–292.
8. Melewicz, F. M., and H. L. Spiegelberg. 1980. Fc receptors for IgE on a subpopulation of human peripheral blood monocytes. *J. Immunol.* **125:** 1026–1031.
9. Boltz-Niţulescu, G., and H. L. Spiegelberg. 1981. Receptors specific for IgE on rat alveolar and peritoneal macrophages. *Cell. Immunol.* **59:** 106–114.
10. Boltz-Niţulescu, G., J. Plummer, and H. L. Spiegelberg. 1982. Fc receptors for IgE on mouse macrophages and macrophage-like cell lines. *J. Immunol.* **128:** 2265–2268.
11. Joseph, M., A. B. Tonnel, A. Capron, and C. Voisin. 1980. Enzyme release and superoxide anion production by human alveolar macrophages stimulated with immunoglobulin E. *Clin. Exp. Immunol.* **40:** 416–422.
12. Joseph, M., J. P. Dessaint, and A. Capron. 1977. Characteristics of macrophage cytotoxicity induced by IgE immune complexes. *Cell. Immunol.* **34:** 247–258.
13. Parkhurst, A. S., A. Burkhalter, and V. H. Cohn, Jr. 1959. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127:** 182–186.
14. Spiegelberg, H. L., and F. M. Melewicz. 1980. Fc receptors specific for IgE on subpopulations of human lymphocytes and monocytes. *Clin. Immunol. Immunopathol.* **15:** 424–433.
15. Davies, G. E., and G. R. Stark. 1970. Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. *Proc. Natl. Acad. Sci. USA.* **66:** 651–656.
16. Segal, D. M., J. Taurog, and H. Metzger. 1977. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci. USA.* **74:** 2993–2997.
17. Lynch, S. M., K. F. Austen, and S. I. Wasserman. 1978. Release of arylsulfatase A but not B from rat mast cells by noncytolytic secretory stimuli. *J. Immunol.* **121:** 1394–1399.
18. Graham, R. C. Jr., and M. J. Karnovsky. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14:** 291–302.
19. Pestel J., M. Joseph, J. P. Dessaint, and A. Capron. 1981. Macrophage triggering by aggregated immunoglobulins. I. Delayed effect of IgG aggregates or immune complexes. *J. Immunol.* **126:** 1887–1891.
20. Melewicz, F. M., L. E. Kline, A. B. Cohen, and H. L. Spiegelberg. 1982. Characterization of Fc receptors for IgE on human alveolar macrophages. *Clin. Exp. Immunol.* **49:** 364–370.
21. Czarnetzki, B. M., and H. Behrendt. 1981. Studies on the *in vitro* development of rat peritoneal mast cells. *Immunobiology.* **159:** 256–268.
22. Melewicz, F. M., R. S. Zeiger, M. H. Mellon, R. D. O'Connor, and H. L. Spiegelberg. 1981. Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. *J. Immunol.* **126:** 1592–1595.
23. Ackerman, N. R., J. R. Beebe, and G. Schidlovsky. 1977. Lysosomal enzyme release from guinea pig alveolar cells: biochemical and morphological observations. *Cytobios.* **20:** 7–20.
24. Heise, E. R., and Q. N. Myrwik. 1967. Secretion of lysozyme by rabbit alveolar macrophage *in vitro*. *J. Reticuloendothel. Soc.* **4:** 510–523.
25. Horwitz, A. L., J. A. Kelman, and R. G. Crystal. 1976. Activation of alveolar macrophage collagenase by a neutral protease secreted by the same cell. *Nature (Lond.).* **264:** 772–774.
26. Rodriguez, R. J., R. R. White, R. M. Senior, and E. A. Levine. 1977. Elastase release from human alveolar macrophages: comparison between smokers and nonsmokers. *Science (Wash. DC).* **198:** 313–314.
27. Arnoux, B., D. Duval, and J. Benveniste. 1980. Release of platelet activating factor (PAF-acether) from alveolar macrophages by the calcium ionophore A23187 and phagocytosis. *Eur. J. Clin. Invest.* **10:** 437–441.
28. Rankin, J. A., M. Hitchcock, W. W. Merrill, and P. W. Askenase. 1981. Slow-reacting substance (SRS): IgE-dependent release from alveolar macrophage. *Fed. Proc.* **40:** 1014*a*.(Abstr.)
29. Arnoux, B., J. Durand, M. Rigaud, R. Masse, and J. Benveniste. 1981. Arachidonic acid metabolites in the rat alveolar macrophages. *Am. Rev. Respir. Dis.* **123:** 50*A*. (Abstr)
30. Sirois, P. 1980. Release of mediators from free bronchoalveolar cells: effect of ionophore A23187 and antigen challenge. *J. Immunopharmacol.* **2:** 543–551.
31. Sirois, P., M. Rola-Pleszczynski, and R. Bégin. 1980. Phospholipase A activity and prostaglandin release from alveolar macrophages exposed to asbestos. *Prostaglandins Med.* **5:** 31–47.
32. Vargaftig, B. B., J. Lefort, M. Chignard, and J. Benveniste. 1980. Platelet-activating factor induces a platelet-dependent broncho-constriction unrelated to the formation of prostaglandin derivatives. *Eur. J. Pharmacol.* **65:** 185–192.
33. Valone, F. H., M. Franklin, F. F. Sun, and E. J. Goetzl. 1980. Alveolar macrophage lipoxygenase products of arachidonic acid: isolation and recognition as the predominant constituents of the neutrophil chemotactic activity elaborated by alveolar macrophages. *Cell. Immunol.* **54:** 390–401.
34. Vargaftig, B. B., J. Lefort, A. V. Prancan, M. Chignard, and J. Benveniste. 1979. Platelet-lung *in vivo* interactions: an artefact or a multipurpose model? *Haemostasis.* **8:** 171–182.